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Review

Stability-indicating capillary zone electrophoresis method for the assessment of recombinant human granulocyte-macrophage colony-stimulating factor and its correlation with reversed-phase liquid chromatography method and bioassay

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ABSTRACT

A stability-indicating capillary zone electrophoresis (CZE) method was validated for the analysis of granulocyte-macrophage colony-stimulating factor (rhGM-CSF) using leuprorelin acetate (LA), as internal standard (IS). A fused-silica capillary (75 μm i.d.; effective length, 72 cm) was used at 25 °C; the applied voltage was 12 kV. The background electrolyte solution consisted of 50 mM di-sodium hydrogen phosphate solution at pH 8.8. Injections were performed using a pressure mode at 50 mbar for 9 s, with detection by photodiode array detector set at 200 nm. Specificity and stability-indicating capability were established in degradation studies, which also showed that there was no interference of the excipients. The method was linear over the concentration range of 2.5–200 $\mu g\,mL^{-1}$ ($r^2 = 0.9995$) and the limit of detection (LOD) and limit of quantitation (LOQ) were 0.79 $\mu g\,mL^{-1}$ and 2.5 $\mu g\,mL^{-1}$, respectively. The accuracy was 99.14% with bias lower than 1.40%. The method was applied to the quantitative analysis of biopharmaceutical formulations, and the results were correlated to those of a validated reversed-phase LC method (RP-LC), and an *in vitro* bioassay, showing non-significant differences ($p > 0.05$).

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1. Introduction

Granulocyte-macrophage colony stimulating factor is a cytokine that regulates the proliferation and differentiation of hematopoietic progenitor cells and activates mature granulocytes and macrophages. Human granulocyte-macrophage colony stimulating factor, produced by recombinant DNA technology (rhGM-CSF), expressed in *Escherichia coli*, Molgramostim, is now marketed world-wide for clinical use in enhancing hematopoietic recovery after cancer chemotherapy and bone marrow transplantation [1]. The rhGM-CSF regulates the expression of anti-apoptotic genes in neural progenitor cells showing anti-apoptotic activity [2].

The rhGM-CSF molecule is a non-glycosylated polypeptide chain consisting of 127 amino acids, with four cysteine residues which form two disulfide bonds, between Cys^{54}–Cys^{96}, and Cys^{88}–Cys^{121}. The molecular weight is approximately 14.5 kDa [3,4].

The biological assay based on the proliferation of the factor-dependent cell line TF-1 (ATCC CRL-2003), has been used for the potency assessment of rhGM-CSF. Growth promoting activity has been evaluated by incubation with tetrazolium bromide (MTT) or alamarBlue and measuring absorbance [5–7].

Three enzyme-linked immunosorbent assay (ELISA) have been described and compared to the cell proliferation bioassay for their ability to quantify non-glycosylated rhGM-CSF present in mixtures with variable protein content. This proved to be useful during the production process and was also applied in pharmacokinetic studies [8–10].

Analytical techniques are available to monitor content/potency, purity and chemical stability of pharmaceutical proteins obtained through recombinant technology. No single technique can satisfactorily provide sufficient information about the protein and therefore a combination of physicochemical, immunological and biological methods is recommended [11,12]. Liquid chromatography (LC) has been successfully applied in correlation studies between physicochemical and biological assays, in attempts to find methods for characterization and for monitoring the stability of biotherapeutics [13–15]. However, the development of analytical methods for the analysis of biotechnology-derived products present some difficulties, due to the low dose of the microheterogeneous protein present relative to the large amounts of human serum albumin which is added to prevent adsorption of the protein to the vial walls and to increase stability during storage [16,17].

The reversed-phase liquid chromatography (RP-LC) method offers a high level of accuracy and sensitivity for the analysis of closely related protein variants or degradation products which may have reduced activity and altered immunogenicity [11,18]. However, one drawback of this method is that proteins are usually denatured or dissociated as they are adsorbed on to the column matrix, and, therefore, it is not appropriate for use as a single technique for establishing the potency of preparations which contain non-covalent oligomers and polymers [19]. The rhGM-CSF expressed in *E. coli* was characterized by fast atom bombardment mass spectrometry combined with RP-LC. The fractions of the enzymatic digests were separated by RP-LC using a TSK-ODS column with detection at 230 nm [20]. Also samples of pharmaceutical formulations were analyzed by a gradient RP-LC method using a C_4 column (250 mm × 4.6 mm i.d.), run at a flow rate of 1 mL min^{-1}, and using photodiode array (PDA) detection at 214 nm [21].

Capillary electrophoresis (CE) has expanded its scope as a powerful analytical technique for pharmaceutical analysis, allowing the determination of the active pharmaceutical ingredients and their impurities, with some advantages related to the existing methodologies [22,23]. Samples of rhGM-CSF were analyzed by capillary electrophoresis, capillary isoelectric focusing and gradient RP-LC, demonstrating the effect of salt concentration in the dosage form, on quantitation, reproducibility and efficiency of capillary electrophoresis [24]. The monopegylated rhGM-CSF was characterized by SE-LC and RP-LC, capillary electrophoresis and mass spectrometry, showing the application of these techniques [6]. However, validation of the method is essential to show that the procedure is suitable for its intended purpose [25].

The aim of the work described in this article was to develop and validate a capillary zone electrophoresis method for the analysis of non-glycosylated rhGM-CSF; to correlate the results with a validated RP-LC method, and with an *in vitro* cell culture bioassay; to evaluate correlations and advantages for the content/potency assessment of biopharmaceutical formulations, and thus contribute to the development of methods to monitor stability, improve quality control, and thereby assure therapeutic efficacy of biological medicines.

2. Experimental

2.1. Chemicals and reagents

European Pharmacopoeia Certificated Reference Standard (Ph. Eur. CRS) for rhGM-CSF was obtained from the EDQM (Strasbourg, France) and the 1st International Standard for rhGM-CSF, WHO 88/646, was obtained from the National Institute for Biological Standards and Control-NIBSC (Herts, UK). A total of eight batches of Leucocitim, Blausiegel (São Paulo, Brazil) containing 300 μg/vial of rhGM-CSF were identified by numbers from 1 to 8. The samples were obtained from commercial sources within their shelf life period. Analytical grade di-sodium hydrogen phosphate dodecahydrated was acquired from Merck (Darmstadt, Germany). All chemicals used were of pharmaceutical or special analytical grade. For all of the analyses, ultrapure water was obtained using an Elix 3 coupled to a Milli-Q Gradient A10 system Millipore (Bedford, MA, USA). All solutions were degassed by ultrasonication Tecnal (São Paulo, Brazil) and filtered through a 0.22 μm Millex Millipore filter (Bedford, MA, USA).

2.2. Apparatus

CE experiments were performed on an Agilent ^{3D}CE apparatus Agilent Technologies (Waldbronn, Germany) consisting of a photodiode array (PDA) detector, a temperature-controlling system (4–60 °C) and a power supply able to deliver up to 30 kV. CE ChemStation software was used for instrument control, data acquisition and analysis. The pH of the solutions was measured using a pH-meter, Thermo Orion Model 420 (Beverly, MA, USA).

The LC method was carried out on a Shimadzu LC system (Kyoto, Japan) equipped with a SCL-10A_{VP} system controller, a LC-10 AD_{VP} pump, a DGU-14A degasser, a SIL-10AD_{VP} autosampler, and a SPD-M10A_{VP} PDA detector. Peak areas were integrated automatically by computer using a Shimadzu Class VP® V 6.14 software program.

2.3. Solutions

Working standard and sample solutions of rhGM-CSF were prepared daily by diluting the reference standard and the sample of pharmaceutical formulation in water, to a final concentration of 50 μg mL^{-1}. A reference solution was also diluted to a final concentration of 1 mg mL^{-1} in water. The stock solutions were stored at 2–8 °C protected from light and daily diluted with water to an appropriate concentration and filtered through a 0.22 μm membrane filter. The sample solution was injected and the amount of the biomolecule calculated against the reference standard.

2.4. Electrophoretic procedure

All experiments were carried out on a fused-silica capillary with 75 μm i.d. and 80.5 cm of total length (effective length 72 cm), thermostatized at 25 °C, and detection by PDA set at 200 nm. At the beginning of each working day, the capillary was conditioned by rinsing with 1 mol L^{-1} hydrochloric acid for 5 min, followed by water for 2 min and 1 mol L^{-1} sodium hydroxide for 10 min, and then with a running electrolyte solution for 5 min. To improve the reproducibility of the migration time between injections, the capillary was conditioned again with water (2 min), and a running BGE solution (4 min). Samples were injected using the pressure mode at 50 mbar for 9 s with a constant voltage of 12 kV (current about 54.5 μA) applied during the analysis. Since electrolysis can change the electroosmotic flow (EOF) and affect the migration time, efficiency and selectivity, the running electrolyte was replaced by a fresh solution after each three injections. The BGE solution consisted of 50 mM di-sodium hydrogen phosphate dodecahydrate at pH 8.8, adjusted by adding 8.5% phosphoric acid.

2.5. Reversed-phase LC method

The validated gradient RP-LC method is described elsewhere [21]. Briefly, the elution was carried out on a reversed-phase Phenomenex (Torrance, USA) Jupiter C_4 column (250 mm × 4.6 mm i.d., with a particle size of 5 μm and pore size of 300 Å) maintained at 45 °C. A security guard holder was used to protect the analytical column. The elution was performed using a linear gradient at a constant flow rate of 1 mL min^{-1} and using photodiode array (PDA) detection at 214 nm. Mobile phase A consisted of 0.1% trifluoroacetic acid (TFA) and mobile phase B was acetonitrile with 0.1% TFA, run as follows: time 0 to 0.1 min 37% of B; from 0.1 to 34 min linear up to 50% of B; from 34.01 to 35 min linear down to 37% of B, maintained up to 40 min. The mobile phases were filtered through a 0.22 μm membrane filter Millipore (Bedford, MA, USA). The injection volume was 50 μL for both the reference standard and the samples.

2.6. In vitro cell culture bioassay

The bioassay was performed as described elsewhere [7,21], and the growth-promoting activity of rhGM-CSF assessed on TF-1 cell line (ATCC number CRL-2003). The cells were maintained in culture medium RPMI 1640 containing 10% (v/v) fetal bovine serum Sigma–Aldrich (St. Louis, MO, USA), adding concentrations of GM-CSF (1–20 ng mL^{-1}) for cell proliferation in 75 cm^2 flasks for 24 h, seeding at approximately 2.0–6.0 × 10^5 cells mL^{-1}. The assay was performed in triplicate, the cells were seeded in 96-well microplates BD Biosciences (San Jose, CA, USA) at a density 4 × 10^5 cells mL^{-1} (2 × 10^4 cells/well) and dosed on seeding with two-fold dilution series range starting with 65 IU mL^{-1} (6.5 ng mL^{-1}) of rhGM-CSF. The WHO (88/646) rhGM-CSF was used as standard and the negative control was RPMI 1640 culture medium. Briefly, the plates were incubated at 37 °C, 5% CO_2 for a minimum of 24 h. Then 25 μL/well of MTT solution (5 mg mL^{-1}) was added and the plates were incubated for a further 5 h. Following the addition of 100 μL/well of sodium dodecyl sulfate (240 mg mL^{-1}) overnight, the absorbance was assessed at 595 nm, using microplate reader Thermo Scientific Multiskan FC (Vantaa, Finland).

2.7. Validation of the capillary zone electrophoresis method

The method was validated using samples of a pharmaceutical formulation of rhGM-CSF with a label claim of 300 μg/vial by determinations of the following parameters: specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), robustness, stability, and system suitability test, following the International Conference on Harmonization (ICH) guidelines [25]. Leuprorelin acetate (LA) was used as an internal standard to compensate for any injection errors and minor fluctuations of the migration time, thus improving the reproducibility of the CZE method.

2.8. Forced degradation studies

The stability-indicating capability of the CZE method was determined by subjecting a reference standard solution (50 μg mL^{-1}) and a pharmaceutical formulation (50 μg mL^{-1}) to accelerated degradation by different acidic, basic, oxidative, photolytic and temperature conditions [26]. Working solutions prepared in 1 mol L^{-1} hydrochloric acid were used for acidic hydrolysis, and working solutions in 0.1 mol L^{-1} sodium hydroxide for the basic hydrolysis evaluation. Both solutions were maintained at room temperature for 2 h (refluxed at 100 °C for 6 h, cooled) and neutralized with acid or base, as necessary. Oxidative degradation was induced by storing the solutions in 10% hydrogen peroxide, at ambient temperature for 24 h, protected from light. Photodegradation was induced by exposing the sample in a photostability chamber to 200 W h m^{-2} of near ultraviolet light from 1 to 24 h. For a study under neutral condition, the reference and sample solutions were diluted in water and heated at 70 °C for 2 h. The solutions were diluted with the electrolyte solution to final concentrations of 50 μg mL^{-1}. The interference of the excipients of the pharmaceutical formulation was determined by the injection of a sample containing only a placebo (in-house mixture of all the formulation excipients), and by the standard addition method, where a calibration curve was constructed by the addition of known amounts of the reference substance to the placebo [27]. Then, the specificity of the method was established by determining the peak purity of rhGM-CSF in the samples using a PDA detector.

2.9. Analysis of rhG-CSF in biopharmaceutical formulations

For the quantitation of rhGM-CSF in biopharmaceutical formulations, the respective stock solutions were diluted to an appropriate concentration of 50 μg mL^{-1} with a BGE solution, or a mobile phase, respectively, for the electrophoretic and chromatographic methods, injected in triplicate and the percentage recoveries calculated against the reference substance.

3. Results and discussion

3.1. Optimization of the electrophoretic conditions

To develop the CZE method, some electrolyte solutions containing ammonium phosphate, di-sodium tetraborate, sodium acetate, potassium phosphate, tris (hydroxymethyl) aminomethane, boric acid, respectively, were tested by selecting di-sodium hydrogen phosphate. The optimum pH of a BGE solution containing 50 mM di-sodium hydrogen phosphate dodecahydrate was investigated in the range of 7–11 (Fig. 1), allowing for buffering capacity, but due to the size of the molecule, pH 8.8 was selected since it show better peak symmetry (about 0.98). The di-sodium phosphate dodecahydrate was evaluated at concentrations of 25–100 mM at pH 8.8 (Fig. 2), which demonstrated a significant effect on the separation performance through its influence on the EOF and the current produced in the capillary. A 50 mM solution was selected due to its low effect on current and non-significant increase on the migration time. The effects of organic modifiers, acetonitrile or methanol, in the concentration range of 5–20%, were also evaluated, but no improvement on the electrophoretic conditions was achieved. The

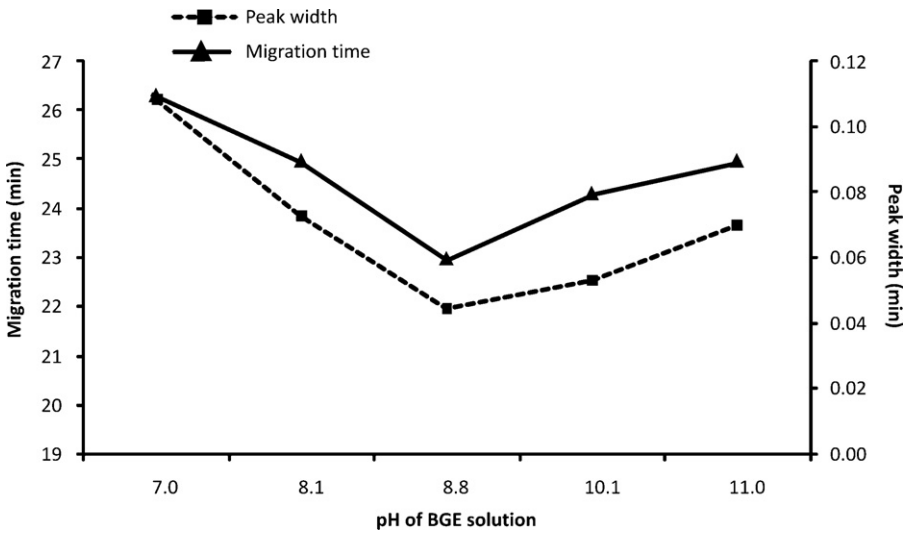

Fig. 1. Effect of pH on migration time and peak width of rhGM-CSF. Running buffer, 50 mM di-sodium hydrogen phosphate dodecahydrate; capillary, 75 μm i.d. × 80.5 cm; applied voltage, 12 kV; UV detection, 200 nm; column temperature, 25 °C. rhGM-CSF 50 μg mL^{-1}, pressure injection at 50 mbar for 9 s.

temperature effect on the separation was investigated in the range of 20–35 °C, and a temperature of 25 °C was chosen due to the short run time and acceptable current. The effect of the voltage was studied through changes from 10 to 30 kV, showing that a potential of 12 kV yielded a short analysis time with an acceptable current (about 54.5 μA), as result of the combination of parameters. Sample solutions were injected using a pressure mode at 50 mbar for 9 s. Wavelength detection was evaluated in the range of 190–400 nm, and a wavelength of 200 nm was chosen due to better sensitivity and signal-to-noise ratio.

3.2. Validation of the method

The stability-indicating CZE method was validated for the analysis of rhGM-CSF in biopharmaceutical formulations with a migration time of about 21.5 min, as shown in the typical electropherogram (Fig. 3a). The acidic condition showed decrease of the area, and only one peak was detected at 20.3 min (Fig. 3b). The basic condition resulted in a decrease of the rhGM-CSF area with one additional peak at 20.7 min (Fig. 3c). The forced oxidative degradation studies exhibited one peak related to the hydrogen peroxide at 19.2 min and additional peaks at 21.2, 23.2, 23.9 and 24.5 min (Fig. 3d). Under the neutral hydrolysis, and the photolytic conditions described, decrease of the areas were observed, respectively, without any additional peak. The specificity of the method was established by determining the peak purity of the analyte and the IS in the working reference substance solution, by overlaying the spectra captured at the apex, upslope and downslope using a PDA detector. Additionally, the standard addition method was applied to evaluate the interference from formulations excipients. The regression equation was determined as $y = 0.0249x - 0.0004$, ($r^2 = 0.9995$), where, x is the concentration of rhGM-CSF, expressed in μg mL^{-1}, and y is the peak-area ratio of rhGM-CSF to IS. No significant difference was found between the slopes calculated for the calibration curve and the standard addition method. The data, together with the peak purity index in the range of 0.9999–1, showed that the peak was free from any co-migrating peak, with no interference of excipients, thus confirming that the proposed method is specific for the analysis of rhGM-CSF.

The linearity was determined by constructing three calibration curves, each one with eight concentrations of rhGM-CSF reference solution in the 2.5–200 μg mL^{-1} range, spiked with leuprorelin acetate at 40 μg mL^{-1}. The value of the determination coefficient calculated by least-squares regression analysis ($r^2 = 0.9995$, $n = 8$,

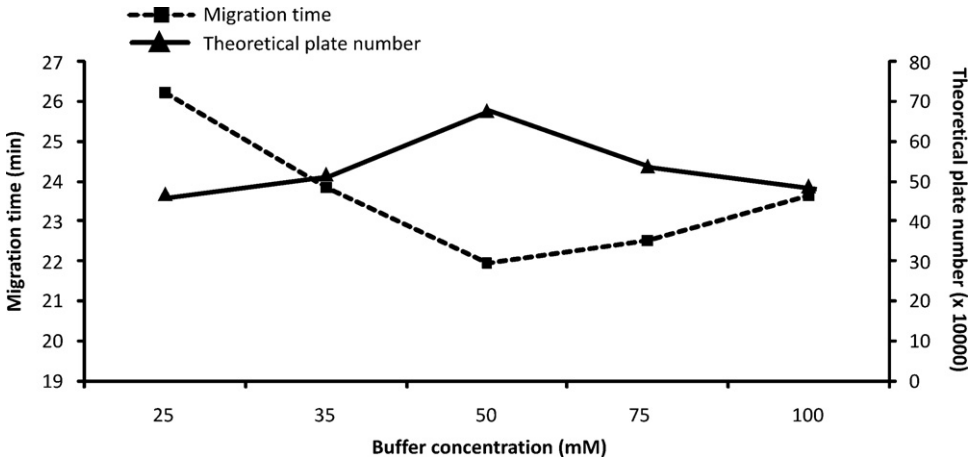

Fig. 2. Effect of buffer concentration on migration time and theoretical plate number of rhGM-CSF. Running buffer, di-sodium hydrogen phosphate dodecahydrate (pH 8.8). Other conditions were as described in the caption of Fig. 1.

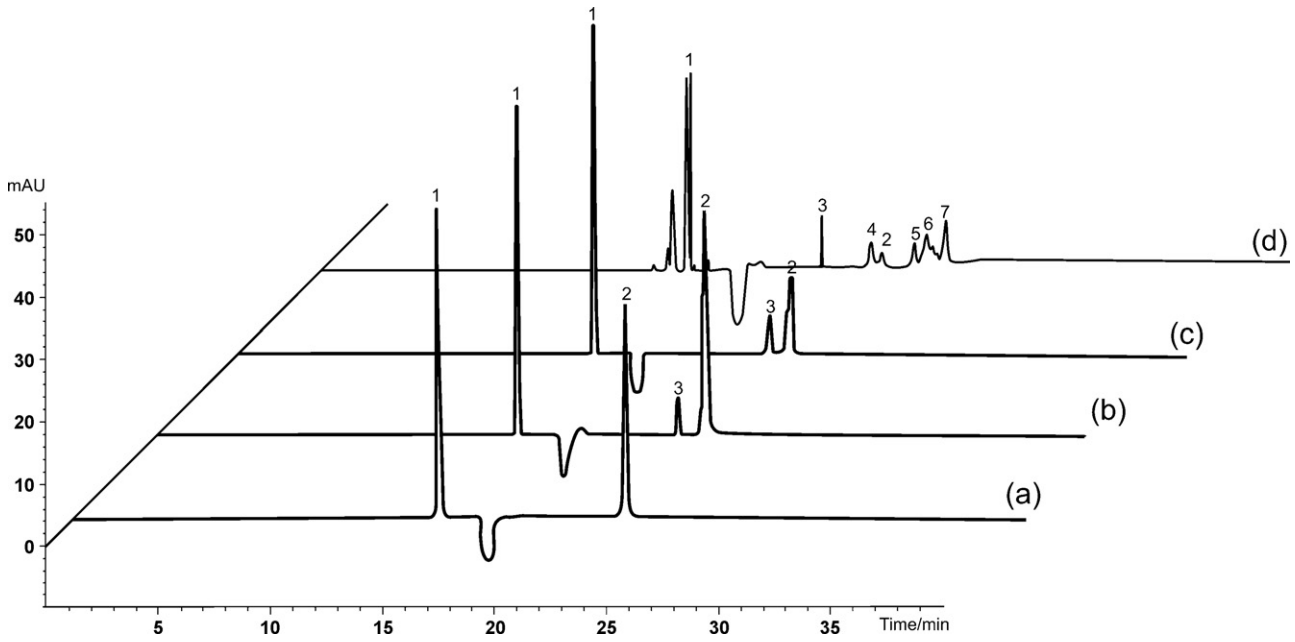

Fig. 3. Representative CZE electropherograms showing peak 1 = leuprorelin (IS) and peak 2 = rhGM-CSF of: (a) Ph. Eur. Reference standard (50 μg mL^{-1}) and IS, and after degradation under: (b) acid hydrolysis, peak 3 = degraded form, (c) basic hydrolysis, peak 3 = degraded form, (d) oxidative condition, peak 3 = hydrogen peroxide, peaks 4, 5, 6, and 7 = degraded forms.

$y = (0.0249 \pm 0.0004)x + (0.0556 \pm 0.0066)$, where, x is concentration in μg mL^{-1} and, y is the peak-area ratio of rhGM-CSF to IS, indicated linearity of the calibration curve for the method.

The precision of the method was evaluated by calculating the relative standard deviation (RSD%) of the migration time and the peak-area ratio, for eight determinations at a concentration of 50 μg mL^{-1}, performed on the same day and under the same experimental conditions. The obtained RSD values were 0.93 and 1.54% for the migration time and the peak-area ratio, respectively. The intermediate precision was assessed by analyzing two samples of the biopharmaceutical formulation on three different days (inter-days) giving RSD values of 1.67 and 1.23%, respectively. The between-analysts precision was determined by calculating the RSD for the analysis of two samples by three analysts; the values were calculated as 1.33 and 1.61%, respectively, as given in Table 1.

The accuracy was assessed from three replicate determinations of three solutions of in-house mixtures of the excipients with known amounts of the biomolecule, containing 40, 50, and 60 μg mL^{-1}. The absolute means obtained with a mean value of 99.14% and a bias lower than 1.40% as given in Table 2, show that the method is accurate within the desired range [28].

The LOD and the LOQ were calculated from the slope and the standard deviation of the intercept determined by a linear-regression model, by using the mean values of the three independent calibration curves. The obtained values were 0.79 and 2.66 μg mL^{-1}, respectively. The evaluated experimental LOQ with a precision lower than 5% and an accuracy within ±5%, [29,30] was determined as 2.5 μg mL^{-1}, and therefore, suitable as an alternative for quality-control analysis. The low sample injection volume and the short optical path-length can be related to the lower sensitivity of the CZE method compared to the chromatographic method.

The robustness of the analytical procedure [31,32] was determined by analyzing samples of the rhGM-CSF reference solution containing 50 μg mL^{-1} in triplicate by the one-variable-at-a-time (OVAT) approach. The results and the experimental range of the selected variables evaluated are given in Table 3, together with the optimized values. Additionally, the robustness was also evaluated and compared by the multi-variable-at-a-time (MVAT) approach

Table 2
Accuracy of CZE for rhGM-CSF in samples of formulations.

Nominal concentration (μg mL^{-1})	Mean concentration found[a] (μg mL^{-1})	RSD[b] (%)	Accuracy (%)	Bias[c] (%)
40	39.44	1.66	98.60	−1.40
50	49.59	2.06	99.18	−0.82
60	59.79	1.91	99.65	−0.35

[a] Mean of three replicates.
[b] RSD = relative standard deviation.
[c] Bias = [(measured concentration − nominal concentration)/nominal concentration] × 100.

Table 1
Inter-days and between-analysts precision data of CZE for rhGM-CSF in samples of biopharmaceutical formulations.

Sample	Inter-days			Between-analysts		
	Days	Concentration found[a] (%)	RSD[b] (%)	Analysts	Concentration found[a] (%)	RSD[b] (%)
	1	98.24		A	100.51	
1	2	100.45	1.67	B	103.75	1.33
	3	99.33		C	101.66	
	1	101.19		A	103.91	
2	2	99.77	1.23	B	103.89	1.61
	3	99.63		C	102.32	

[a] Mean of three replicates.
[b] RSD = relative standard deviation.

Table 3
CZE conditions and range investigated during robustness testing with the one-variable-at-a-time (OVAT) procedure.

Variable	Range	rhGM-CSF[a] (%)	RSD	Migration time	RSD	Symmetry	RSD	Optimized condition
Electrolyte solution pH	8.2	98.10	0.98	22.03	0.77	0.87	0.41	
	8.4	98.40	1.01	21.90	0.68	0.84	0.43	
	8.6	98.55	1.11	21.96	0.42	0.85	0.34	8.8
	8.8	99.18	0.54	21.56	0.35	0.98	0.28	
	9.0	99.03	0.97	21.88	0.80	0.83	0.75	
Electrolyte solution concentration (mM)	48	102.11	1.63	22.65	0.86	0.94	0.92	
	50	100.15	0.34	21.64	0.48	0.96	0.58	50
	52	100.87	0.79	22.02	1.12	0.87	0.74	
Temperature (°C)	23	99.02	0.87	22.35	1.03	1.06	0.27	
	25	100.55	0.45	21.66	0.85	0.97	0.16	25
	27	99.21	0.56	22.03	0.97	1.08	0.38	
Voltage (kV)	10	97.18	1.52	22.08	0.46	1.02	0.78	
	12	99.36	0.78	21.30	0.42	0.97	0.65	12
	14	98.05	1.14	22.69	0.66	0.92	0.97	
Time injection (s)	8	101.11	0.26	21.90	1.09	1.05	0.49	
	9	100.71	0.09	21.63	1.05	1.01	0.18	9
	10	98.15	1.04	21.94	1.21	0.96	0.55	
Wavelength (nm)	198	100.85	1.82	21.89	1.24	0.88	1.05	
	200	98.45	1.10	21.78	1.04	0.95	0.88	200
	202	98.17	1.29	21.87	1.12	0.89	0.97	

[a] Mean of three replicates.

at three levels (1 unit per parameter up or down around optimized values) [31]. This procedure gives results for minimum changing of the maximum number of parameters at a time, and is a very useful, rapid and efficient approach for a robustness determination. The results for the OVAT and MVAT procedures were within the acceptable deviation (RSD < 2%), and an analysis of the variance showed non-significant differences ($p > 0.05$) for the dosage of the sample solutions. Moreover, the peak symmetry values were also evaluated, showing non-significant differences ($p > 0.05$). The electropherogram pattern was not altered and different capillary batches also indicated robustness under the conditions tested.

The stability of rhGM-CSF in BGE was assessed after storage of the samples for 48 h at 2–8 °C, and also placed into the auto-sampler for 24 h at room temperature, showing non-significant changes (<2%) relative to freshly prepared samples, as previously indicated [33].

A system suitability test was carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using five replicate injections of a reference standard solution containing 50 μg mL^{-1} of rhGM-CSF. The obtained RSD values for the migration time, peak area, peak symmetry and peak width were 0.06%, 1.79%, 1.22%, and 1.53%, respectively. The number of theoretical plates was approximately 58,357, with RSD of 1.07%. The parameters tested were within the acceptable range (RSD < 2%).

3.3. Method application

The CZE method was applied to the determination of rhGM-CSF in biopharmaceutical formulations and the results compared to those obtained using a validated RP-LC method and an *in vitro* bioassay, giving mean differences of the estimated content/potency of 1.01% lower, and of 2.77% higher, respectively, as shown in Table 4. The experimental values were compared statistically by analysis of the variance (ANOVA), which showed non-significant differences ($p > 0.05$). The potential demonstrated by the proposed method will be useful in the determination of rhGM-CSF without prior separation of the excipients of the formulation, with the added

Table 4
Comparison between the electrophoresis, and chromatography method, and bioassay in an assay of rhGM-CSF in biopharmaceutical formulations.

Sample	CZE[a]	RP-LC[a]		*In vitro* bioassay[a]	
	(%)	Main peak (%)	Deamidated/sulfoxides (%)	Potency (%)	Confidence intervals ($p = 0.95$)
1	96.16	98.57	1.17	97.63	94.78–100.47
2	98.61	100.18	1.25	94.87	92.02–97.71
3	101.99	103.15	2.03	94.79	91.94–97.63
4	91.68	92.57	0.76	92.45	89.69–95.20
5	99.24	98.89	1.26	97.10	94.34–99.94
6	106.56	107.68	0.84	106.15	103.39–108.90
7	100.54	99.97	0.72	95.89	93.04–98.73
8	99.78	101.65	1.63	93.48	90.63–96.32
Mean	99.32	100.33	1.21	96.55	–
SD	4.03	4.02	0.42	3.97	–
			ANOVA		
			Between-methods		*F* calculated[b]
					1.67

[a] Mean of three replicates.
[b] *F* critic for $p = 0.05$.

advantages of small sample volumes without the consumption of organic solvents, and a short analysis time.

4. Conclusions

The results of the validation studies show that the CZE method is sensitive with a LOQ of 2.5 $\mu g\,mL^{-1}$ accurate with a mean value of 99.14%, possesses significant linearity ($r^2 = 0.9995$) and precision characteristics without any interference from the excipients. Therefore, the method can be applied as an alternative with advantages for the biotechnology process and through subsequent purification steps. This will enable the monitoring of the stability and assure the batch-to-batch consistency of the bulk and finished rhGM-CSF in biopharmaceutical formulations of the biotechnology-derived medicine.

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