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Stability-indicating capillary zone electrophoresis method for the assessment of recombinant human interleukin-11 and its correlation with reversed-phase liquid chromatography and biossay

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ABSTRACT

A stability-indicating capillary zone electrophoresis (CZE) method was validated for the analysis of recombinant human interleukin-11(rhIL-11) using rupatadine fumarate, as internal standard (IS). A fused-silica capillary, (50 μm i.d.; effective length, 40 cm) was used at 25 °C; the applied voltage was 20 kV. The background electrolyte solution consisted of 50 mmol L^{-1} sodium dihydrogen phosphate solution at pH 3.0. Injections were performed using a pressure mode at 50 mbar for 45 s, with detection by photodiode array detector set at 196 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 1.0–300 μg mL^{-1} (r^2=0.9992) and the limit of detection (LOD) and limit of quantitation (LOQ) were 0.2 μg mL^{-1} and 1.0 μg mL^{-1}, respectively. The accuracy was 100.4% with bias lower than 1.1%. Moreover, the *in vitro* cytotoxicity test of the degraded products showed significant differences ($p < 0.05$). The method was applied for the content/potency assessment of rhIL-11 in biopharmaceutical formulations, and the results were correlated to those of a validated reversed-phase LC method (RP-LC) and an TF-1 cell culture assay, showing non-significant differences ($p > 0.05$). In addition the CZE and RP-LC methods were applied for the analysis of rhIL-11 in human plasma. Therefore, the proposed alternative method can be applied to monitor stability, to assure the batch-to-batch consistency and quality of the bulk and finished biotechnology-derived medicine.

1. Introduction

Thrombocytopenia occurs in patients with malignancies undergoing myelosuppressive chemotherapy, which may lead to hemorrhagic tendency and treatment delay. Recombinant human interleukin-11 (rhIL-11) is a kind of cytokine produced by DNA technology in *Escherichia coli*, now marketed worldwide as Oprelvekin, for clinical use in prevention of severe chemotherapy-induced thrombocytopenia and to reduce the need for platelet transfusions in patients with nonmyeloid malignancies [1–3].

The rhIL-11 biomolecule consists of 177 amino acids polypeptide chain, non-glycosylated with a molecular mass of 19 kDa and isoelectric point of 11.7. Differs from the naturally occurring human interleukin-11 (IL-11) only by the absence of an amino-terminal proline, and the presence of two residues of Met^{58} and Met^{122} [4].

The bioassays are useful to assess the efficacy and quality of those proteins, which cannot be adequately characterized only by physicochemical methods. Early signals triggered by IL-11 were assessed in a multifactor-dependent human erythroleukemic cell line TF1, which showed that this protein stimulated cell proliferation [5] and was applied to assess the bioactivity of biopharmaceuticals formulations [6]. However, a major concern of using a bioassay is its precision, which is generally inferior to the precision of physicochemical techniques [7–9].

Physicochemical techniques are used to monitor content/potency, purity, chemical stability of biopharmaceutical proteins obtained through recombinant DNA technology. No single technique can satisfactorily provide sufficient information about the protein and therefore a combination of physicochemical, immunological, and biological methods is recommended, and has been applied in correlation studies [10–13]. 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 [14,15]. A linear gradient RP-LC method using C_4

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column and UV detection at 214 nm was employed to determine the oxidative effect of plastic tubes used for storage of protein samples [16]. Optimal storage stability of lyophilized rhIL-11 was evaluated by quantifying the oxidation levels and cleavage products by the gradient RP-LC [17]. A stability-indicating RP-LC method was validated using a C_4 column with PDA detection at 214 nm, and was applied to the assessment of rhIL-11 in biopharmaceutical formulations [6]. Capillary electrophoresis (CE) has expanded its scope as a powerful analytical technique for pharmaceutical analysis, allowing the determination of biotechnology-derived medicines and their degraded forms, charged variants and isoforms [9,10,18–20]. At the moment, rhIL-11 is not included in any Pharmacopoeia and no CE method has been published for quality control analysis. However, validation of the method is essential to show that the procedure is suitable for its intended purpose [21].

The aim of this research was to develop and validate a specific, sensitive and stability-indicating capillary zone electrophoresis (CZE) method for the analysis of rhIL-11; to correlate the results with a validated RP-LC method and with an *in vitro* bioassay; and to evaluate the bioactivity and the cytotoxicity of the degraded forms, thus contributing to the development of an alternative method to monitor stability, improve quality control, and thereby assuring the therapeutic efficacy of the biotechnology-derived medicine.

2. Experimental

2.1. Chemicals and reagents

Reference reagent Interleukin-11, human rDNA derived, (*R-rhIL*-11 WHO 92/788), for bioassays was obtained from the National Institute for Biological Standards and Control-NIBSC (Herts, UK). Biological reference substance of rhIL-11, (*BRS-rhIL*-11), for physicochemical assays was supplied by Amoytop Biotech Co., Ltd. (Xiamen, Fujian, China). Rupatadine fumarate (IS) was purchased from by Sequoia Research Products (Oxford, UK). A total of ten batches of Plaquemax® Bergamo (São Paulo, Brazil), containing 5 mg/vial of rhIL-11 were identified by numbers from 1 to 10 and two batches of Neumega® Wyeth (São Paulo, Brazil), containing 5 mg/vial of rhIL-11 were identified by numbers from 11 to 12. Samples were obtained from commercial sources within their shelf life period. Acetonitrile, disodium hydrogen phosphate, glycine, methanol, sodium dihydrogen phosphate, sodium dodecyl sulfate and trifluoroacetic acid used as reagents or excipients were purchased from Merck (Darmstadt, Germany). Fetal bovine serum, RPMI-1640 medium and thiazolyl blue formazan (MTT) were acquired from Sigma-Aldrich (St. Louis, MO, USA). 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).

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. The 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 RP-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. Capillary zone electrophoresis method

2.3.1. Solutions preparation

Stock solutions were prepared by diluting the *BRS-rhIL*-11 reference solution and the sample of biopharmaceutical formulation in water, to a final concentration of 400 μg mL^{-1}. IS was diluted to a final concentration of 200 μg mL^{-1} in methanol. The stock solutions were stored at 2–8 °C protected from light and daily diluted with BGE to working concentrations of 40 μg mL^{-1} and 20 μg mL^{-1} respectively, for the rhIL-11 and IS, and filtered through a 0.22 μm membrane Millex Millipore (Bedford, MA, USA).

2.3.2. Electrophoretic procedure

All experiments were carried out on a fused-silica capillary with 50 μm i.d. and 48.5 cm of total length (effective length 40 cm), thermostatized at 25 °C, and using a PDA detector set at 196 nm. At the beginning of each working day, the capillary was conditioned by rinsing with 1 mol L^{-1} sodium hydroxide for 5 min, followed by water for 2 min and 1 mol L^{-1} phosphoric acid for 5 min, and then by water for 2 min and with a running BGE solution for 5 min. Samples were injected using the pressure mode at 50 mbar for 45 s with a constant voltage of 20 kV (current about 55.2 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 Background electrolyte solution (BGE) consisted of 50 mmol L^{-1} sodium dihydrogen phosphate at pH 3.0, adjusted by adding 8.5% phosphoric acid.

2.3.3. Validation of the capillary zone electrophoresis method

The method was validated using samples of a biopharmaceutical formulation of rhIL-11 with a label claim of 5 mg/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 ICH guidelines [21,22]. Rupatadine fumarate (IS) was selected as internal standard to compensate for any injection errors and minor fluctuations of migration time, thus improving the reproducibility and performance of the CZE method.

2.3.4. Forced degradation studies

The stability-indicating capability of the CZE method was determined by subjecting a *BRS-rhIL*-11 reference solution (400 μg mL^{-1}) and a biopharmaceutical formulation (400 μg mL^{-1}) to accelerated degradation by different acidic, basic, oxidative, photolytic and temperature conditions [23,24]. Working solutions prepared in 1 mmol L^{-1} hydrochloric acid were used for acidic hydrolysis and working solutions in 1 mmol L^{-1} sodium hydroxide for the basis hydrolysis evaluation. Both solutions were maintained at room temperature for 10 min and 1 h, respectively, and neutralized with base or acid, as necessary. Oxidative degradation was induced by maintaining the solutions in 3% hydrogen peroxide, at ambient temperature for 3 min, 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, sample solutions were diluted in water and heated at 80 °C for 3 h. Then, the solutions were diluted with the BGE solution to final concentrations of 40 μg mL^{-1}. The interference of the excipients of the biopharmaceutical 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