Journal of Chromatography B, xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Method development and qualification of capillary zone electrophoresis for investigation of therapeutic monoclonal antibody quality

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 4 July 2016
Accepted 14 July 2016
Available online xxx

Keywords:
Capillary zone electrophoresis
Method development
Therapeutic monoclonal antibody
Biotechnology
Charge variants

ABSTRACT

Capillary electrophoresis techniques are widely used in the analytical biotechnology. Different electrophoretic techniques are very adequate tools to monitor size—and charge heterogenities of protein drugs.

Method descriptions and development studies of capillary zone electrophoresis (CZE) have been described in literature. Most of them are performed based on the classical *one-factor-at-time* (OFAT) approach. In this study a very simple method development approach is described for capillary zone electrophoresis: a "**two-phase-four-step**" approach is introduced which allows a rapid, iterative method development process and can be a good platform for CZE method. In every step the current analytical target profile and an appropriate control strategy were established to monitor the current stage of development. A very good platform was established to investigate intact and digested protein samples.

Commercially available monoclonal antibody was chosen as model protein for the method development study. The CZE method was qualificated after the development process and the results were presented. The analytical system stability was represented by the calculated RSD% value of area percentage and migration time of the selected peaks (<0.8% and <5%) during the intermediate precision investigation.

1. Introduction

Monoclonal antibodies are the most emerging classes of therapeutic biosimilar protein drugs. Their overall characterization is a critical in the point of the patients' safety. General papers were published recently focusing on the comprehensive comparability of biosimilar candidates to the originator [1,2].

Monoclonal antibodies are very heterogeneous molecules in both size and charge. Antibody characterization and homogenity assessment require a huge analytical arsenal of separation techniques [3,4]. For primer sequence and microheterogenity determination the mass spectrometry coupled liquid chromatography or capillary electrophoresis has the major importance [5,6]. Secondary and tertiary structure determination is performed by different spectroscopic methods such as fluorescence or circular dichroism as far as infrared spectroscopy-based methodology [7–9].

Charge variant analysis has big impact among the routine analytical methods since certain type of chemical or biological modifications have influence on the biological activity of the antibody. Such modifications like deamidation of asparagines may have influence on biological activity of the molecule [10,11].

Many basic laboratory techniques are used in research laboratories to determine charge variant distribution [12–14]. Techniques like gel-based isoelectric focusing provide very good resolution but time- and buffer-consuming and very hard to use in regulated environment. Capillary isoelectric focusing also provide very good resolution and give adequate information about the isoelectric points of each charge variants [15,16], but the analysis is relatively slow (takes about 30–50 min for each runs) and very sensitive for the way of sample preparation because its complexity.

In routine analytical applications the different separation techniques are the most commonly used. Mainly strong cation exchange chromatograpic techniques play the most important role in quality control processes of therapeutic MABs regarding the charge heterogeneity assessment [17].

Among them the capillary zone electrophoresis is one of the most popular tools for protein characterisation. This is a very good

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http://dx.doi.org/10.1016/j.jchromb.2016.07.026
1570-0232/

methodology for a rapid, cost-effective analysis of therapeutic proteins [18,19,22]. Different background electrolyte systems were described in previous studies. Shi et al. developed and validated method based on 20 mM acetate–acetic acid (at pH 6.0) with the addition of 0.3% polyethylene oxide (PEO) and 2 mM triethylenetetramine (TETA) [19]. He et. al developed a CZE method for charge variant analysis based on 600 mM ε-aminocaproic acid (EACA) buffer at pH 5.4 and 0.1% HPMC as polymeric additive [18].

Most recently an intercompany study of capillary zone electrophoresis method for charge hetereogenity analysis of monoclonal antibodies was published by Moritz et. al [20]. This paper enhances the advantages of EACA system in an international precision study, where repeatability was <1% within all of the companies.

In this study a novel and very simple method development strategy for capillary zone electrophoresis methods is introduced to investigate a commercially available monoclonal antibody drug product as a model protein. EACA buffer system was chosen as background electrolyte. Our goal was to develop an analytical method which can be a good platform both for "top-town" and "middle-up" analysis of monoclonal antibodies. I order to establish a fast and simple method development strategy the "two-phase-one-step" approach was introduced, which could be easily adopted into any biopharmaceutical laboratory.

Our aim was also to adjust a proper method which is capable of resolving the main isoforms of a commercially available antibody chosen for this study as model protein. Method qualification was also performed to prove that the developed method is serves its purpose. Linearity, level-of-detection (LOD), level-of-quantification (LOQ), intermediate precision and specificity were tested. Effects of oxidation and deamidation on the charge profile of the investigated monoclonal antibody were also studied during specificity testings.

2. Materials and methods

2.1. Materials

Hydroxi-propyl methyl cellulose (HPMC), triethylenetetramine (TETA), ε-aminocaproic acid (EACA), hydrogen peroxide, phosphoric acid, papain enzyme, Carboxypeptidase-B (CPB) enzyme, Tris(hydroxymethyl)aminomethane (TRIS), hydrogen peroxide, methionine, ammonium hydrogen carbonate and phosphoric acid were purchased form Sigma (St Louis, USA, Missouri). Commercially available monoclonal antibody was purchased from Janssen Biotechnology Inc. (Horsham, USA, Pennsylvania). Amicon Ultra 3 K Centrifugal Filter system was purchased from Merck Millipore (Darmstadt, Germany)

2.2. Instrumentation

All of the measurements were performed on Beckman Coulter(Hercules, USA Califonia) PA800 and PA800 Plus capillary electrophoresis systems. 50 μm inner-diameter fluorocarbon coated capillaries (μSil-FC) were purchased from Agilent Technologies (Santa Clara,USA California) The polyimide coating onthe both ends of the capillaries were removed by Microsolv CE Window Maker apparatus (Microsolv, USA Monmouth). Hettich Mikro 200 benchtop centrifuge (Andreas Hettich GmbH & Co.KG, Germany Tuttlingen)

2.3. Buffers and solutions

100 mM phosphoric acid was used as cleaning solution during capillary cleaning. 25 mM TRIS ph 7.0 was used for papain enzymatic reaction. 25 mM, 50 mM and 100 mM EACA buffers was made by diluting stock EACA solution with purified water.

2.4. Sample solutions

Papain digested sample solution contained monoclonal antibody in 1 mg/ml concentration diluted with 20 mM TRIS pH 7.0 solution the digestion was performed during 2.5 h at 37 °C. 200 μl solution was containing 4 μl papain enzyme.

Intact antibody solution was used in 10 mg/ml concentration, where the original formulation buffer was removed and the protein was replaced into CZE running buffer by Amicon Ultra 10 K filtration unit after spinning 30 min at 14000 g speed.

2.5. Deamidated samples

10 mg/ml intact antibody solution was incubated for 30 h at room temperature in 0.1 M ammonium hydrogen carbonate solution. The buffer of the solution was removed and the protein was replaced into CZE running buffer by Amicon Ultra 10 K filtration unit after spinning 30 min at 14000 g speed.

2.6. Oxidized samples

10 mg/ml intact antibody solution was incubated for 90 min at room temperature with 1 v/v% 1 M H2O2 solution. The oxidation procedure was stopped by adding methionine to the reaction mixture. The buffer of the solution was removed and the protein was replaced into CZE running buffer by Amicon Ultra 10 K filtration unit after spinning 30 min at 14000 g speed.

2.7. General zone electrophoresis setup

The basic setup were not modified during method development were the following: injection was performed during 10 s with 0.5 psi hydrodinamical pressure. 30 kV separation voltage was applied during all of the measurements in 50 cm long (40 cm effective length) coated capillary. Before daily use the capillary was flushed by purified water for 5 min at 50 psi, for 5 min with 100 mM phosphoric acid at 50 psi, followed by a 5 min rinse with purified water at 50 psi. In every single run a 5 min-long water flushing was used at 50 psi in the beginning and at the end of the method.

3. Results and discussions

3.1. Method development strategy (MDS)

In our study a simple '*two-phase and four-step*' approach was introduced (Table 1) which can speed up the time of method developments of CZE systems. Two phase means a fast *screening phase*, where the pH and the ionic strength are being adjusted to receive the optimal migration time stability and the most optimal resolving capacity of the method, and a *fine tuning phase* where the amount of organic additives and viscosity enhancers are adjusted to be optimal to reach the maximum in peak resolving with the maximum migration time and area percentage stability. Some physical parameters (separation voltage, injection time and pressure, capillary length) were not investigated since the choice of buffer system (EACA, TETA, HPMC) was based on the literature available. To minimize electroosmotic flow and minimize protein precipitation a fluorocarbon coated capillary was chosen. This choice was based on costs and availability on the market.

Please cite this article in press as: D. Suba, et al., Method development and qualification of capillary zone electrophoresis for investigation of therapeutic monoclonal antibody quality, J. Chromatogr. B (2016), http://dx.doi.org/10.1016/j.jchromb.2016.07.026