Journal of Pharmaceutical and Biomedical Analysis 132 (2017) 133–140

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Design of a strong cation exchange methodology for the evaluation of charge heterogeneity in glatiramer acetate

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ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form
27 September 2016
Accepted 2 October 2016
Available online 4 October 2016

Keywords:
Validation
SCX-UPLC
Glatiramer acetate
ATP
UPLC method

ABSTRACT

Complex pharmaceuticals are in demand of competent analytical methods able to analyze charge heterogeneity as a critical quality attribute (CQA), in compliance with current regulatory expectations. A notorious example is glatiramer acetate (GA), a complex polypeptide mixture useful for the treatment of relapsing-remitting multiple sclerosis. This pharmaceutical challenges the current state of analytical technology in terms of the capacity to study their constituent species. Thus, a strong cation exchange methodology was designed under the lifecycle approach to support the establishment of GA identity, trough the evaluation of its chromatographic profile, which acts as a charge heterogeneity fingerprint. In this regard, a maximum relative margin of error of 5% for relative retention time and symmetry factor were proposed for the analytical target profile. The methodology met the proposed requirements after precision and specificity tests results, the former comprised of sensitivity and selectivity. Subsequently, method validation was conducted and showed that the method is able to differentiate between intact GA and heterogeneity profiles coming from stressed, fractioned or process-modified samples. In summary, these results provide evidence that the method is adequate to assess charge heterogeneity as a CQA of this complex pharmaceutical.

1. Introduction

The analysis of complex pharmaceuticals or their process intermediates containing heterogeneous mixtures of proteins or peptides demands the use of specific and precise separation techniques. In this sense, certain analytical methodologies including mass spectrometry, electrophoresis and chromatography have been designed to evaluate quality attributes during the lifecycle of a drug [1–7]. However, the analysis of non-biological complex drugs, such as glatiramoid peptides, is scarce and remains as a challenging task due to the dispersity degree of their molar masses and sequences distributions (i.e.: intrinsic heterogeneity) [8].

Strong cation exchange chromatography (SCX) is a commonly used analytical technique that separates peptide or protein variants according to their net charge and electric dipole moment. Specifically, trough the analyte differential interaction with a stationary phase that contains negatively-charged aliphatic sulfonic acid groups on its surface [1,9–11].

Traditionally, SCX is performed at acidic pH above 3.0, where the sulfonic acid groups are completely ionized and exhibit maximal ion-capacity [12]. Hence, separation occurs mainly from the differences in the positive charge within the analyzed mixture, being the species with the least positive net charge the first to elute [10]. However, electric dipole moments and charge densities influence the separation as well, as a result of structure-, size-, mass- and sequence-related phenomena for the analyzed species. In effect,

Abbreviations: % RTR, Retention Time Ratio Percent; ATP, Analytical Target Profile; CQA, Critical Quality Attribute; CV, Coefficient of Variation; GA, Glatiramer Acetate; ICH, The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; MP-PALS, Massively Parallel Phase Analysis Light Scattering; NCA, N-carboxyanhydride; PALS., Phase Analysis Light Scattering; R^2., Coefficient of Determination; RME., Relative Margin of Error; RP-UPLC, Reverse Phase - Ultra Performance Liquid Chromatography; RRT, Relative Retention Time; RT, Retention Time; RTs, Sample Retention Time; RTwrs, Working Reference Standard Retention Time; SCX, Strong Cation Exchange Chromatography; SCX-UPLC, Strong Cation Exchange - Ultra Performance Liquid Chromatography; SE-UPLC, Size Exclusion - Ultra Performance Liquid Chromatography; SF, Symmetry Factor; WRS, Working Reference Standard.

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http://dx.doi.org/10.1016/j.jpba.2016.10.002
0731-7085/

the strength of the ionic interactions established by each of the analyzed species against the stationary phase determines their elution order.

Therefore, SCX is useful to assess charge heterogeneity, a critical quality attribute (CQA) known for its role in the molecular recognition and interactions that governs a drug's pharmacological behavior [13]. Further complemented with orthogonal or complementary techniques, SCX can be applied for the characterization and qualification of complex products.

Glatiramer acetate (GA) is a member of the glatiramoids family used for the treatment of relapsing-remitting multiple sclerosis through its immunomodulatory, neuroprotective and neuroregenerative properties [8,14,15]. GA is comprised of a complex co-polymer mixture of L-alanine, L-glutamic acid, L-lysine and L-tyrosine at a specific molar ratio. This pharmaceutical is synthesized from the polymerization reaction of activated amino acids (N-carboxyanhydrides, NCA-amino acids) followed by a partial depolymerization of the intermediate copolymers, resulting in a wide range of peptides with different molecular masses and amino acid sequences [16].

GA's complexity defies the current state of analytical technology towards the establishment of reproducible charge heterogeneity profiles. This becomes more challenging considering the uneven distribution of the charged amino acids (i.e.: lysine and glutamic acid) within its co-polymers. Besides, a proper methodology must be able to detect changes in the characteristic GA heterogeneity distribution, owed to variations within the manufacturing processes or exposure to stressful conditions.

To our best knowledge, a methodology capable to evaluate the charge heterogeneity of GA as an indicative of its identity and stability has not been reported to date. Although, significant advances have been made for the characterization of GA trough distinct techniques, including MS [17], only qualitative or statistical results after an exhaustive examination are reported, which is unpractical for routine analysis. Herein, we propose a fast, specific and reproducible SCX methodology capable to assess the charge heterogeneity of GA polypeptide mixture as a whole, whose distinctive species with characteristic abundances are depicted by a particular chromatographic profile. This methodology was designed according to the lifecycle approach [18–20] to satisfy the necessity to establish criteria that will allow an unequivocal GA discrimination from non-desired heterogeneity profiles in terms of distribution width and elution shifts resulting from manufacturing changes or deviations (from now on referred to as non-desired profiles) when compared to the characteristic GA profile. For this purpose, the method was challenged at two different stages: first, against the established Analytical Target Profile (ATP), including the demonstration of their capabilities to asses charge heterogeneity and to discriminate non-desired profiles with complementary techniques such as massively parallel phase analysis light scattering, reverse phase and size exclusion chromatographies, and second, by the acceptance criteria during method validation, in order to comply with regulatory requirements [18,19].

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used for the analyses were at least ACS grade and were obtained from J.T. Baker (Avantor Performance Materials, Inc.; Center Valley, PA) or Sigma Aldrich (St. Louis, MO). All assays were performed using ultrapure Milli-Q water (Millipore, Billerica, MA).

2.2. Sample preparation

2.2.1. GA samples

Probioglat® drug substance and drug product (GA 20 mg/mL solution for subcutaneous use) were obtained from Probiomed S.A. de C.V. (Mexico City, Mexico) while Copaxone® (GA 20 mg/mL solution for subcutaneous use) was obtained from Teva Pharmaceutical Industries (Central District, Israel).

2.2.2. Working reference standard (WRS)

A characteristic batch of GA drug substance was set as a WRS based on a characterization and standardization exercise performed in accordance with our internal quality management procedures (Probiomed S.A. de C.V.).

2.2.3. UV stressed samples

Samples were diluted in a 4% (w/v) mannitol solution to 2 mg/mL and dispensed into 4.2 mL quartz cells (Beckman Coulter, Brea, CA). Samples were exposed independently to UV light with a radiated power of 2.0 W at 254 nm using Osram Germicidal Puritec HNS G5 lamps (Osram Gmbh, Munich, Germany), during 10, 15 and 20 min. Stressed samples were prepared by triplicate.

2.2.4. Molecular mass fractionated samples

Molecular mass fractionated samples of GA were generated by serial fractionation using modified polyethersulfone cassettes with 50 kDa, 10 kDa and 3 kDa nominal molecular weight cutoffs (Omega Centramate 0.1 SQ M/1.1 SQ.FT) from Pall Corporation (New York, NY). 10 g of lyophilized GA were reconstituted in water to 65 mg/mL and then filtered with a 50 kDa cassette coupled to a Crossflow Filter Holder (Sartorius Stedim Biotech, Aubagne, France). The retained solution was washed with water until the permeate absorbance at 275 nm was lower than 0.05 UA. Collected permeate was used to obtain the subsequent fractions at 10 kDa and 3 kDa using the same procedure.

2.2.5. Process-modified samples

Standardized process conditions established for GA manufacturing were modified in order to generate different heterogeneity distributions as follows:

- Experiment A: Polymerization was initiated with NCA-lysine and NCA-alanine only, after 7 min NCA-tyrosine and NCA-glutamic acid were added, followed with the standard process.
- Experiment B: Polymerization was initiated with NCA-lysine and NCA-alanine only, after 14 min NCA-tyrosine and NCA-glutamic acid were added, followed with the standard process.
- Experiment C: Polymerization was initiated with NCA-glutamic acid and one third of the NCA-lysine and NCA-alanine material only, after 7 min the remaining material along with NCA-tyrosine were added, followed with the standard process.
- Experiment D: Polymerization was initiated with one third of the NCA-lysine and NCA-alanine material only, after 7 min the remaining material along with NCA-glutamic acid and NCA-tyrosine were added, followed with the standard process.
- Experiment E: double amount of NCA-Lysine was used during polymerization, followed with the standard process.

2.3. Strong cation exchange—ultra performance liquid chromatography (SCX-UPLC)

Samples were analyzed by SCX-UPLC using a 7 μm Protein-Pak Hi Res SP 4.6 × 100 mm column on an Acquity H-Class Bio UPLC System, equipped with an inert flow path suited for the high ionic strength aqueous conditions. An Acquity UPLC Fluorescence Detector was used, with excitation and emission wavelengths of 275