Journal of the Neurological Sciences 359 (2015) 24–34

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

Demonstration of equivalence of a generic glatiramer acetate (Glatopa™)

James Anderson [a], Christine Bell [a,*], John Bishop [a], Ishan Capila [a], Tanmoy Ganguly [a], Joseph Glajch [a], Mani Iyer [a], Ganesh Kaundinya [a], Jonathan Lansing [a], Joel Pradines [a], James Prescott [a], Bruce A. Cohen [b], Daniel Kantor [c], Richard Sachleben [a]

[a] Momenta Pharmaceuticals, Inc., 675 West Kendall Street, Cambridge, MA 02142, United States
[b] Northwestern University Feinberg School of Medicine, 303 East Chicago Avenue, Chicago, IL 60611, United States
[c] Kantor Neurology, 4851 West Hillsboro Blvd, Suite A-1, Coconut Creek, FL 33973, United States

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 30 September 2015
Accepted 5 October 2015
Available online 8 October 2015

Keywords:
Multiple sclerosis
Glatiramer acetate
Drugs
Generic
Generic equivalency

ABSTRACT

Glatiramer acetate (GA) has been available under the brand name Copaxone® for nearly two decades. Recently, the US Food and Drug Administration (FDA) approved the first generic GA, Glatopa™, as fully substitutable for all indications for which Copaxone 20 mg is approved; Glatopa also represents the first FDA-approved "AP-rated," substitutable generic for treating patients with MS. Glatiramer acetate is a complex mixture of polypeptides and, consequently, its characterization presented challenges not generally encountered in drug development. Despite its complexity, and without requiring any clinical data, approval was accomplished through an Abbreviated New Drug Application in which equivalence to Copaxone was evaluated across four criteria: starting materials and basic chemistry; structural signatures for polymerization, depolymerization, and purification; physicochemical properties; and biological and immunological properties. This article describes the rigorous overall scientific approach used to successfully establish equivalence between Glatopa and Copaxone, and presents key representative data from several of the comprehensive sets of physicochemical (structural) and biological (functional) assays that were conducted.

1. Introduction

Glatiramer acetate (GA; Copaxone®, Teva Pharmaceuticals USA Inc., North Wales, PA, USA) is approved for the treatment of relapsing forms of multiple sclerosis (MS) [1]. Its mechanism of action is complex and involves immunomodulation of both the innate and adaptive immune systems [2]. Known mechanisms include alteration of regulatory T-cell function [3–5], with induction of a T-helper 1 (Th1) to Th2 cell shift resulting in a more anti-inflammatory cytokine profile [6–10], alteration of antigen-presenting cell (APC) function [3], and modulation of B-cell function [2]. The possible neuroprotective effects of GA, which are mediated by neurotrophic factors, include the ability to reduce demyelination and promote remyelination [2]. Glatiramer acetate is considered a disease-modifying therapy that is dosed as a baseline immunomodulatory agent administered for extended periods of time [11,12]. Glatiramer acetate and similar therapies are indicated to reduce the frequency of exacerbations in patients with relapsing MS [1,12–16], and are commonly initiated when patients show signs of relapsing MS [12].

Due to the widespread use and relatively high cost of Copaxone, there is increasing interest in the development of generic versions of GA to reduce costs and increase access to this medication for patients with MS. Recently, the US Food and Drug Administration (FDA) approved the first generic GA, Glatopa™, which has been approved as a fully substitutable generic for all indications for which Copaxone 20 mg is approved [17].

In the United States, a generic drug is approved under an Abbreviated New Drug Application (ANDA). Using this process, preclinical or clinical data are generally not required to establish safety and effectiveness of the generic drug [18]. Instead, generics must demonstrate therapeutic equivalence (i.e., pharmaceutical equivalence and bioequivalence) to the innovator drug product [19]. Bioequivalence is defined by the Code of Federal Regulations, 21CFR320.1, as the "absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives

Abbreviations: ANDA, Abbreviated New Drug Application; APC, antigen-presenting cell; CNS, central nervous system; DEA, diethylamine; EAE, experimental autoimmune encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; FDA, Food and Drug Administration; GA, glatiramer acetate; MHC, major histocompatibility complex; mAbs, monoclonal antibodies; MS, multiple sclerosis; MOG, myelin oligodendrocyte glycoprotein; NCAs, N-carboxyanhydrides; PLP, proteolipid peptide.

* Corresponding author.
E-mail addresses: janderson@momentapharma.com (J. Anderson), cbell@momentapharma.com (C. Bell), jbishop@momentapharma.com (J. Bishop), icapila@momentapharma.com (I. Capila), tganguly@momentapharma.com (T. Ganguly), jglajch@momentapharma.com (J. Glajch), miyer@momentapharma.com (M. Iyer), ganesh@momentapharma.com (G. Kaundinya), jlansing@momentapharma.com (J. Lansing), jpradines@momentapharma.com (J. Pradines), jprescott@momentapharma.com (J. Prescott), bac106@northwestern.edu (B.A. Cohen), dkantor.md@gmail.com (D. Kantor), rsachleben@momentapharma.com (R. Sachleben).

http://dx.doi.org/10.1016/j.jns.2015.10.007

becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study [19]." Per Code of Federal Regulations 21CFR320.22 [20], and as is routinely determined for injectable products, the bioequivalence of Glatopa has been deemed self-evident based on the fact that it is a parenteral solution intended solely for administration by injection and that it contains the same active and inactive ingredients in the same concentrations as the approved drug product, Copaxone. Although GA is a mixture of polypeptides, it is produced by a completely chemical synthesis and is not a biologic; therefore, the ANDA process was the appropriate regulatory pathway.

Establishing pharmaceutical equivalence involves demonstrating "sameness" of the active ingredient, as well as other product characteristics, such as dosage form and concentration. While the latter qualities are easily verified, demonstrating "sameness" of the active ingredient requires both detailed characterization of the active ingredient and development of a process that reproducibly yields an equivalent material. Most generic medicines approved to date consist of small-molecule, one-component active ingredients, where the demonstration of sameness can be achieved through first-principle chemical analysis and the chemical connections can be fully ascribed. The development of the process to produce a generic is typically designed to make the correct end product, while minimizing the levels of side-products and impurities. These processes need not be similar to the brand process, and the characterization of the process is not a critical component of the demonstration of sameness of the active ingredient. While the size and complexity of the generics approved in recent years has increased, most generics still follow this simple model of proof of structure and quality via direct chemical analysis.

Glatiramer acetate, however, is a complex mixture of synthetic polypeptides with a range of molecular weights and sequences, manufactured from the copolymerization of the amino acids L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in a specific molar ratio [1,6,10]. Therefore, due to the large number of possible components, structural elucidation by complete component analysis is not possible. However, the exact analysis of each component is neither practical nor necessary, and the equivalence of Glatopa to Copaxone was demonstrated by a comprehensive set of physicochemical and biological characterization techniques, in combination with a thorough understanding of the process used to make GA. The combination of thorough product characterization and process understanding was utilized to achieve and demonstrate equivalence of the GA in Glatopa and Copaxone, the reference listed drug.

2. Materials and methods

A strategy was developed to establish equivalence based on an understanding of the chemistry, manufacturing process, and biology of GA. The following four-point criteria were employed for evaluating the equivalence of Glatopa to Copaxone.

- Equivalence of starting materials and basic chemistry
- Equivalence of structural signatures for polymerization, depolymerization, and purification
- Equivalence of physicochemical properties
- Equivalence of biological and immunological properties

Using this framework, this article describes the process of establishing sameness and presents data demonstrating the equivalence of Glatopa to Copaxone 20 mg.

3. Results and discussion

3.1. Characterization of the reference listed drug

To develop an equivalent product and process, a thorough understanding of the brand product was first required. This was accomplished by a review of the available scientific, patent, and regulatory literature on Copaxone and by extensive physicochemical, biological, and immunological characterization of Copaxone. These characterizations involved the use of more than 60 methods, with up to 50 different Copaxone lots being measured for some attributes. In addition to describing Copaxone, the use of multiple-lot testing served to measure and express the diversity and range of the commercial lots of Copaxone for certain quantitative attributes. The data from these analyses are presented as examples of the application of the four equivalence criteria, which are discussed in the following sections.

3.2. Equivalence of starting materials and basic chemistry

The chemical process used to manufacture the drug substance for Copaxone is relatively straightforward and well understood; the basic framework (i.e., identity of reagents, solvents, ratios, and processing steps) have been available in the public literature for many years [21]. It consists of three basic chemical steps, followed by a final purification step (Fig. 1): 1) polymerization of four amino acid N-carboxyanhydrides (NCAs) initiated by diethylamine; 2) depolymerization and deprotection of the initially formed protected polypeptide mixture; and 3) final deprotection of the second intermediate polypeptide mixture, followed by purification and counter ion-exchange.

The same starting materials (NCAs) with the same protecting groups are used for the manufacture of both Glatopa and Copaxone. Similarly, the same solution-phase polymerization, HBr-based depolymerization, and deprotection chemistry are applied in the synthesis of both Glatopa and Copaxone. The identities of the starting materials, reagents, and solvents have been previously described [1,22] and were confirmed through the detection of residual materials, such as protecting groups in the brand product (i.e., Lys[TFA] and Glu[OBn]). The starting materials were extensively investigated with several conventional analytical methods (i.e., spectroscopic, chromatographic, and chiral purity and impurity analysis), and the effects of the starting material quality were assessed (e.g., the impact of NCA impurities on product attributes).

3.3. Equivalence of structural signatures for polymerization, depolymerization, and purification

As noted, GA is a complex mixture of polypeptides and its characterization presents challenges not generally encountered in generic drug development. However, while GA is complex, it is not complicated and is produced by a process that is well documented, well understood, and decipherable. Consequently, the impact of process conditions on specific product attributes can be elucidated experimentally. The product attributes that are directly attributable and sensitive to the processes of polymerization, depolymerization, and purification are referred to as *process signatures*. As part of the development of generic GA, a thorough understanding of this process was developed by coupling the analysis of GA process signatures with process development. The demonstration of the equivalence of process signatures for the three steps in both the Glatopa and Copaxone processes ensures that the processes used to manufacture the two materials are equivalent.

It should be noted that process conditions need not be identical to produce equivalent material. In fact, extensive process characterization studies were performed to not only discover the process signatures but to also define the acceptable ranges for all critical process conditions (e.g., reaction temperatures, concentration of reactants) that influenced the corresponding process signatures. The total evaluation comprised developing the analytical methods appropriate for measuring process signatures specific to each of the chemical steps used to produce GA, and defining the range of process conditions that produce equivalent and nonequivalent process signatures. Therefore, comparing different lots of GA on the basis of process signatures can demonstrate whether or not equivalent processes were used to produce the materials. GA

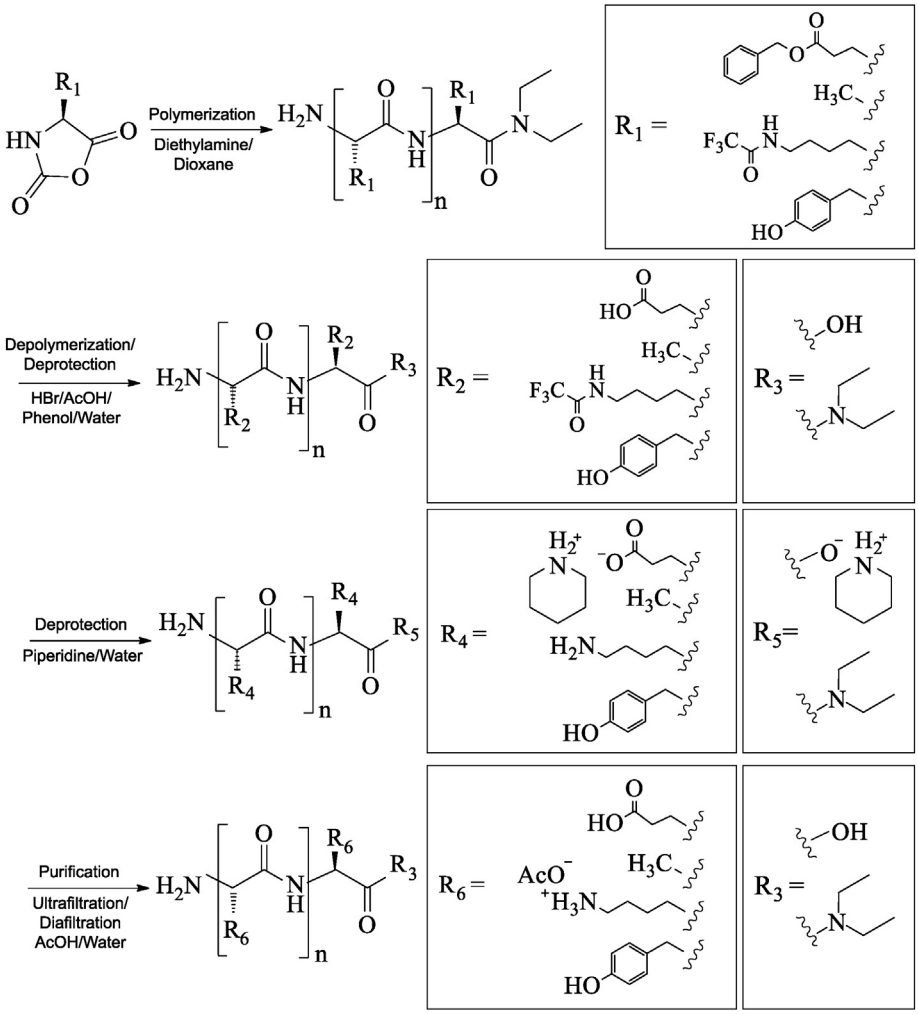


Fig. 1. Synthetic scheme for glatiramer acetate.

produced by equivalent processes is by definition pharmacologically equivalent.

Structural signatures for each process step were developed through understanding of the chemistry and extensive mathematical modeling of differential reaction kinetics. Each process signature was demonstrated to have measurable and consistent effects on the final product. Some structural signatures selected as being representative of each step of the manufacturing process are shown in Table 1.

One example of how process conditions create process signatures in the final product is the effect of composition drift, a concept that has been described in polymer chemistry since 1930 [23]. Specifically, because the monomers differ in reactivity, the initiation and polymerization reactions consume monomers nonuniformly, causing the reactant proportions to change over the course of the reaction. Thus, the polymer composition varies in a controlled and predictable manner as the polymer chains grow. This composition drift defines the sequences generated by the process.

The process signatures that are due to composition drift are used to characterize the first step of the process in the synthesis of GA. Fig. 2 describes the polymerization reaction that occurs in step 1 of the process. The polymerization is initiated by the reaction of one NCA with diethylamine (DEA) and is propagated by reaction of the N-terminus of that amino acid with a subsequent amino acid. The abundance of amino acids at the beginning of the chain (near the DEA) is governed by the relative amount of each NCA present and the differential reaction kinetics between each NCA and the growing polymer, with the most reactive amino acids dominating. As the chains propagate and NCAs are depleted, the ratio of amino acids available for reaction changes so that the ends of the chains have proportionally more of the less reactive amino acids. This is referred to as composition drift and can be monitored by several process signatures.

For example, the proportion of each amino acid that is adjacent to DEA in the final GA (i.e., as an amino acid diethylamide) is different from the bulk ratio of the amino acids in the product; subsequently, the ratio of amino acid diethylamides can be monitored as a reflection

Table 1
Selected process signatures for each step in the glatiramer acetate process.

Process step	Chemistry	Glatiramer acetate structural signature
1. Polymerization	Reagents/charge	• Amino acid proportions • Total diethylamide content
	Initiation	• Amino acid diethylamide proportions
	Propagation	• N-terminal amino acid sequence
2. Depolymerization/ deprotection	Cleavage preferences	• C-terminal amino acid proportions • N-terminal amino acid proportions • Pyroglutamate content • Molar mass distribution
3. Deprotection/ purification	Removal of small peptides	• Molar mass distribution • Amino acid profile across molar mass distribution

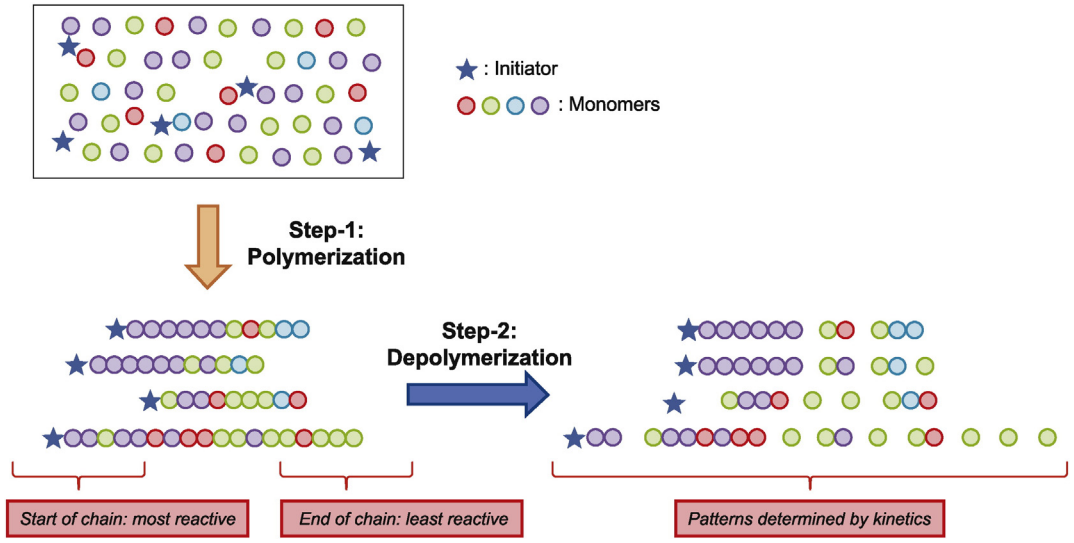


Fig. 2. Pictorial representation of the step 1 N-carboxyanhydride polymerization and step 2 depolymerization of Intermediate-1. The figure demonstrates the composition drift in the intermediate 1 polymer, which is controlled by reaction kinetics, as well as the origin of the C- and N-termini created in step 2, which are controlled by cleavage preferences. These diagrams are illustrative only and do not represent the actual distribution sequence generated in step 1.

of the initiation kinetics of the step 1 reaction. Fig. 3 shows the relative levels of each diethylamide in the final GA for multiple lots of Glatopa and Copaxone. The levels for several lots of Glatopa and Copaxone overlap, demonstrating not only equivalence of the final products but, specifically, equivalence of the step 1 polymerization reaction.

To confirm that the differential reaction kinetics for step 1 do cause a composition drift in the final product, the generation of material under conditions with different kinetics was performed. In one example, the step 1 reaction was performed with a different solvent while the remaining steps were performed as normal. The resulting material was designated as a negative control because it matched the Copaxone label specifications for amino acid composition and peak molecular weight, but was known to have been made with different sequence preferences due to the deliberate change to reaction kinetics. Fig. 3 also includes the results of the analysis for diethylamide content of

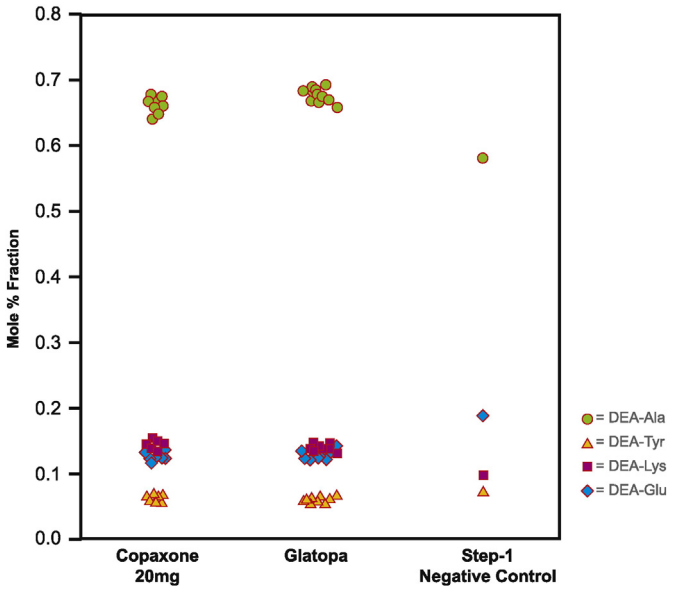


Fig. 3. Relative level of each amino acid adjacent to diethylamine in final glatiramer acetate for several lots of Glatopa, Copaxone, and a step 1 negative control, where the reaction was run in a different solvent and resulted in different relative reaction kinetics. Ala, alanine; DEA, diethylamine; Glu, glutamate; Lys, lysine; Tyr, tyrosine.

the negative control. In this case different amino acid diethylamide proportions are observed, directly due to the different reaction kinetics for the polymer initiation step of the process. Additional negative controls were designed and used to confirm the process signatures described in Table 1. The use of such negative controls proves the sensitivities of the analytical methods employed and validates understanding and control of the process.

In step 2 of the process, the long-chain polypeptides created in step 1 are cleaved to create smaller peptides, and the benzyl protecting group on glutamic acid is removed (Fig. 2). The extent and sites of the cleavages are governed by reaction conditions, such as time and temperature, and also by the cleavage preferences (i.e., kinetics) of individual bonds. These cleavage preferences are reflected in the C- and N-termini observed in the final GA. Fig. 4 shows the relative amino acid levels at the N-termini of GA for the first five cycles of N-terminal analysis by Edman degradation. The results for four representative Glatopa lots were contained within the acceptance criteria based on the observed range for multiple lots of Copaxone. Because the N-terminal characteristics are a combination of the composition drift from step 1 and the cleavage preferences in step 2, the equivalence of this attribute further confirms the equivalence of GA processes.

As with the previous process signature example, negative controls were used to probe the sensitivity of the N-terminal process signature to different reaction conditions. The negative control shown in Fig. 3, which used step 1 conditions where different reaction kinetics were present, was also run by N-terminal analysis and the results are shown in Fig. 4. For this sample, several points are beyond the specification limits, demonstrating the sensitivity of this measure to the step 1 process conditions. Results for a step 2 negative control are also included in Fig. 4. In this sample, the depolymerization conditions were modified, leading to alteration of the cleavage preferences and subsequent changes in the N-terminal profiles.

The amino acids present at the C-termini were similarly examined to demonstrate the equivalence of the step 2 processes. Fig. 5 shows the relative levels of each amino acid at the C-termini that terminate in a carboxylic acid. These are indicative of C-termini that are generated during the step 2 cleavage reaction, as opposed to the C-termini that result from the original initiation reaction and that are present as amino acid diethylamides (Fig. 3). As with the N-termini, the results for four representative Glatopa lots were contained within the acceptance criteria based on the observed range for multiple lots of Copaxone. The step 2 negative control using the alternate depolymerization

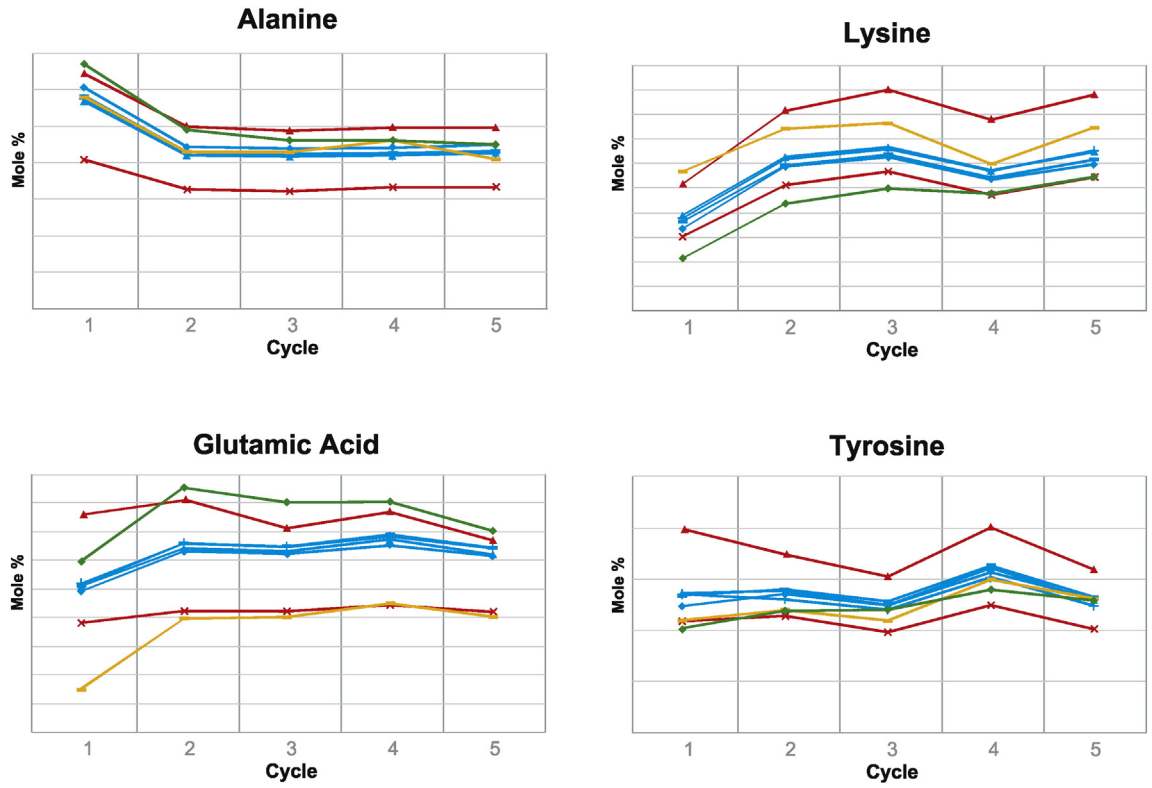


Fig. 4. Relative amino acid levels at the N-termini of glatiramer acetate for the first 5 cycles of N-terminal analysis by Edman degradation. The red lines represent the Copaxone-based specifications for each amino acid for each cycle. Within these lines are the results for 4 representative Glatopa lots (blue). Also shown are 2 negative controls: one is a step 1 reaction negative control (orange) where the reaction was run with a different solvent; the other is a step 2 negative control (green), in which the depolymerization reaction was altered.

conditions was also run by this C-terminal analysis and results at or outside the acceptance ranges were observed for three of the four amino acids, demonstrating the sensitivity of this measure to the process conditions.

Step 3 of the process is a final deprotection of the lysine amino acid followed by purification and final acetate salt exchange. An example of a process signature of this step is the final molar mass distribution. Although the molar mass is in part determined by the step 2 cleavage, the final distribution is governed by step 3, wherein removal of small peptides is achieved via diafiltration. Fig. 6A shows the overlay of the molar mass distribution for lots of Glatopa and Copaxone, demonstrating the equivalence of this attribute in both materials. To demonstrate the sensitivity of this measure to the process, Fig. 6B shows the molar mass distribution for a lot of GA before and after the diafiltration step. The before sample functions as a negative control for this step and comparison of these chromatograms shows that the filtration operation reduces the

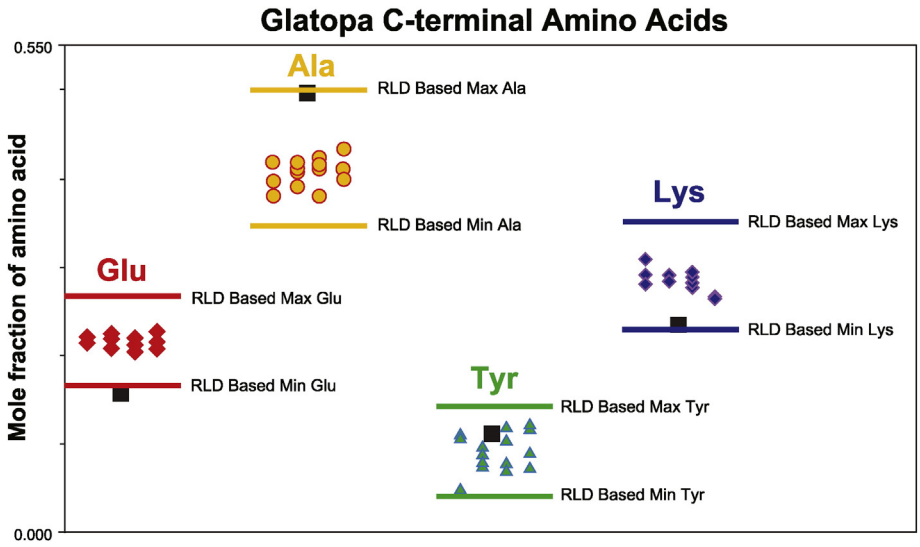


Fig. 5. Relative amino acid levels at the C-termini of glatiramer acetate. The solid lines for each amino acid represent the Copaxone-based specifications for that amino acid. Within these lines are the results for multiple Glatopa lots. Also shown is a step 2 negative control, in which the depolymerization reaction was altered (black squares). Note: this method quantifies the C-termini that end in COOH, and does not include the peptides that terminate in diethylamide (see Fig. 3). Ala, alanine; Glu, glutamate; Lys, lysine; Tyr, tyrosine; RLD = reference listed drug.

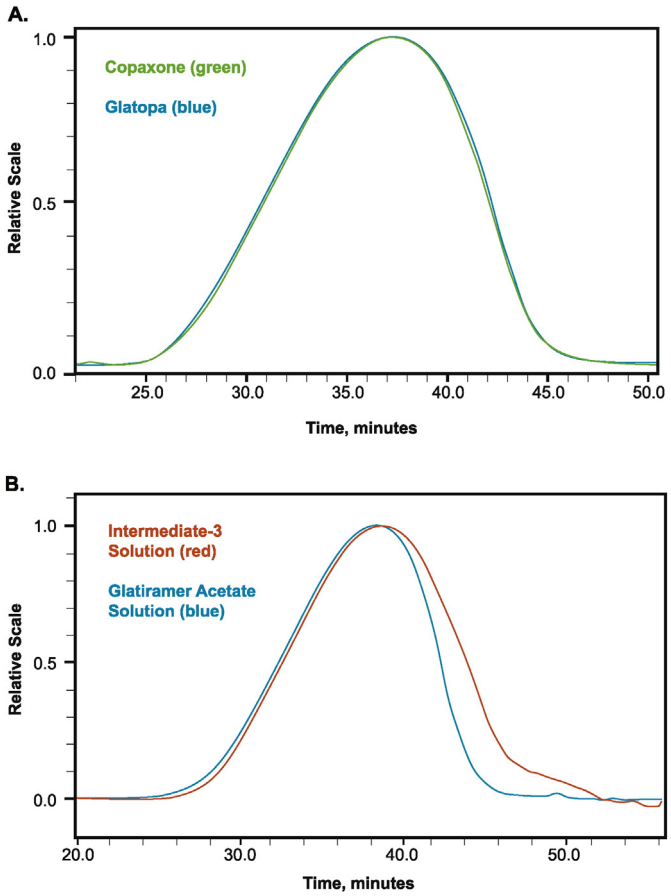


Fig. 6. A) overlay of the molar mass distributions of Glatopa and Copaxone, as measured by size exclusion chromatography. Peptides elute by size, with large peptides eluting first. B) overlay of the molar mass distributions of a lot of glatiramer acetate lot before (the step 3 solution) and after (final GA solution) the diafiltration step.

amount of shorter peptide chains as reflected by the narrowing of the distribution on the right-hand side between the Intermediate-3 solution and the GA solution. While both of these solutions meet the specification for peak molar mass, only the final GA sample gives a polydispersity within the Copaxone-based specification, demonstrating the importance of this process signature to the control and understanding of the process.

A comprehensive suite of methods has been developed to measure the full complement of process signatures, analogous to the examples given herein for each process step. As described, these methods were also performed on negative controls (nonequivalent glatiramoid materials) to confirm their sensitivities to process changes. The measurement of the complete set of process signatures was combined with process design space experiments to achieve a full understanding of each process step and its effect on the final product. This allowed for the establishment of a process for Glatopa that is equivalent to that used to manufacture Copaxone.

3.4. Equivalence of physicochemical properties

In addition to the extensive, innovative, and proprietary approach used to demonstrate the equivalence of process signatures, the methods defined in Section 3.3 form a subset of all the techniques used to study the physicochemical attributes of GA. Approximately 45 different methods were employed to characterize the drug substance and drug product and to ensure the quality of the product. These included many methods that are orthogonal to the ones previously described, but also those that probed additional physicochemical attributes (e.g., higher-order structure, chirality), bulk composition properties

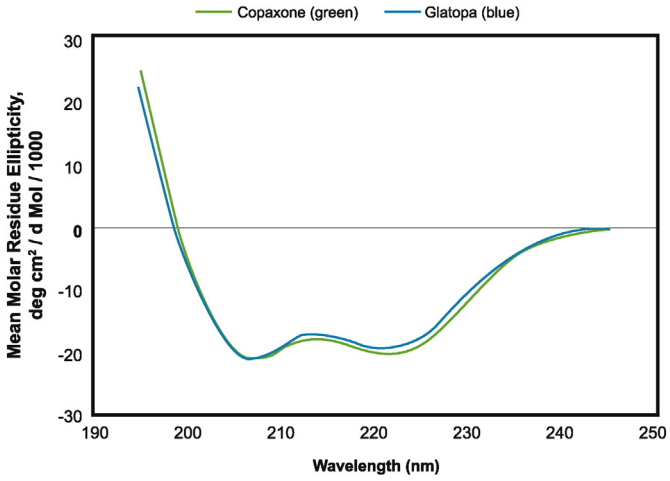


Fig. 7. Overlaid circular dichroism spectra of Glatopa and Copaxone.

(e.g., amino acid content, nuclear magnetic resonance and infrared spectral properties), and many that define general quality attributes (e.g., impurities, concentration, potency).

An example of an additional method is the measure of circular dichroism as an indication of the extent of secondary structure of the polypeptide chains. Fig. 7 shows the spectra of a Copaxone lot and a Glatopa lot, which demonstrate equivalence of the two materials for this attribute. In fact, the level of α-helical content calculated from these spectra was shown to be numerically the same for the two materials. In another example, the total amino acid compositions of numerous lots of Glatopa and Copaxone were shown to be equivalent (Fig. 8).

3.5. Equivalence of biological and immunological properties

Having achieved the physicochemical equivalence of Glatopa to Copaxone by matching the starting materials, process signatures, and physicochemical properties, the biological functions of GA were examined to demonstrate the equivalence of Glatopa to Copaxone in aggregate biological function and in the key aspects of its biology.

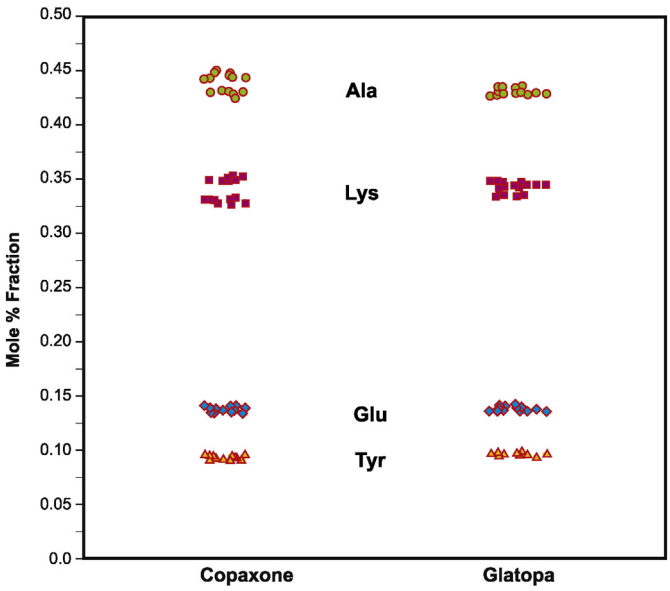
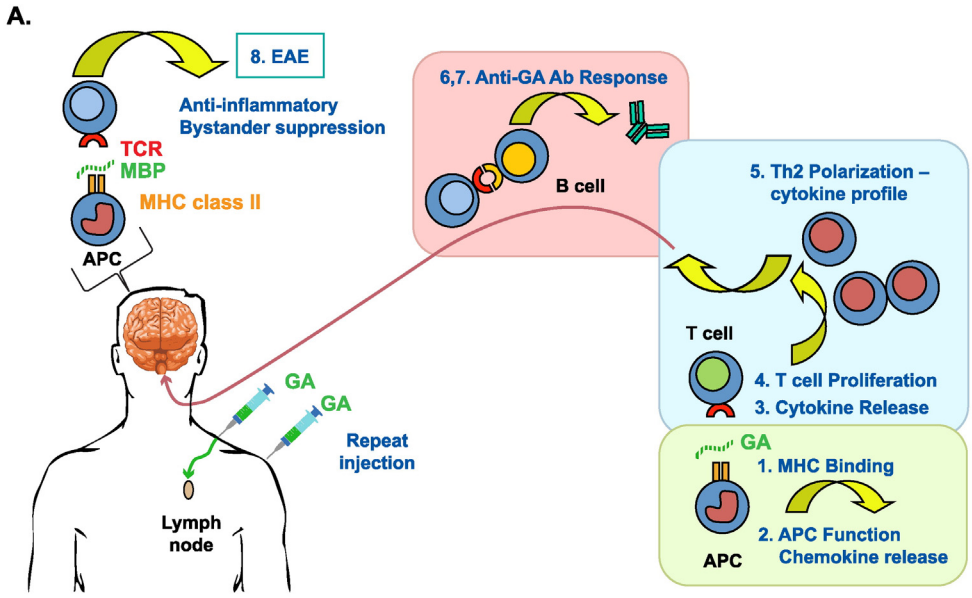


Fig. 8. Amino acid compositions as mole fractions of final glatiramer acetate for several lots of Glatopa and Copaxone. Ala, alanine; Glu, glutamate; Lys, lysine; Tyr, tyrosine.

A.



B.

APC Biology	T-cell Biology	B-cell Biology	Aggregate	Immunorecognition/ Miscellaneous
1. APC function: THP-1 MIG ELISA				
2. T-cell cytokine release: mTh2 polarized T-cell IL-4 ELISA				
3. T-cell polarization: measurement of Th1/Th2 cytokines				
4. Anti-glatiramer acetate antibody response: titer, cross-reactivity and isotype				
5. EAE–PLP/CFA in SJL/J mice, MOG in C57 Bl/6 mice and PLP-based T-cell adoptive tranfer using therapeutic dosing in SJL/J mice				6. and 7. pAb and mAb ID test
				8. mAb sandwich ELISA

Fig. 9. The biological and immunological evaluation of Glatopa vs Copaxone. A) Cells related to the various mechanisms of action of glatiramer acetate (GA). B) Assays covering aggregate biological function and key aspects of GA biology (e.g., T- and B-cell biology). Ab, antibody; EAE, experimental autoimmune encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; mAB, monoclonal antibody; MBP, myelin basic protein; MHC, major histocompatibility complex; TCR, T-cell receptor.

This strategy employed multiple, redundant, and orthogonal biological and immunological assays, including major histocompatibility complex (MHC) II class binding, APC function, T-cell proliferation, T-cell polarization, B-cell biology, antibody response, immunorecognition, genome-wide gene expression effects [24], anti-inflammatory effects, and neuroprotection. Fig. 9 illustrates this comprehensive and rigorous approach, and the following are some examples.

The first assay was based on the ability of GA to modulate the immune system. Following subcutaneous administration, GA was processed by the professional APCs (dendritic cells) and peptide fragments were presented in the context of MHC class II antigen to stimulate T-cell activation. Naive T cells initially responded to GA through polyclonal, antigen-specific proliferation and cytokine production. With repeated exposure to GA, the T-cell response to GA was modulated over time toward a tolerogenic Th2-like phenotype. These GA-reactive Th2-like cells are thought to migrate from the periphery to the central nervous system (CNS) and exert an anti-inflammatory effect on the local pathogenic inflammatory response through the secretion of anti-inflammatory cytokines [2]. The broad (antigen non-specific) suppression of pathogenic cells by GA-specific T cells has been termed bystander suppression [5,25]. Secretion of the soluble Th2 cytokine interleukin-4 (IL-4) from murine GA-specific Th2 polarized cells is a measure of T-cell activation [6]. Two key cell types are required for GA-induced IL-4 secretion: antigen-specific cells (murine splenocytes depleted of T cells) and murine GA-responsive, Th2-polarized T cells [25]. GA-induced IL-4 secretion was quantified using an IL-4 enzyme-linked immunosorbent assay (ELISA), and the relative potency of Glatopa and Copaxone served as a specific measure of biological equivalence. The relative potencies for multiple lots of Glatopa compared to a reference lot of Copaxone (chosen for its potency at the median of a panel of 11 RLD lots) were between 93% and 107%, demonstrating equivalence with respect to this biological response.

Aggregate measures of the efficacy of GA were measured by the use of in vivo animal models. Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model to mimic multiple

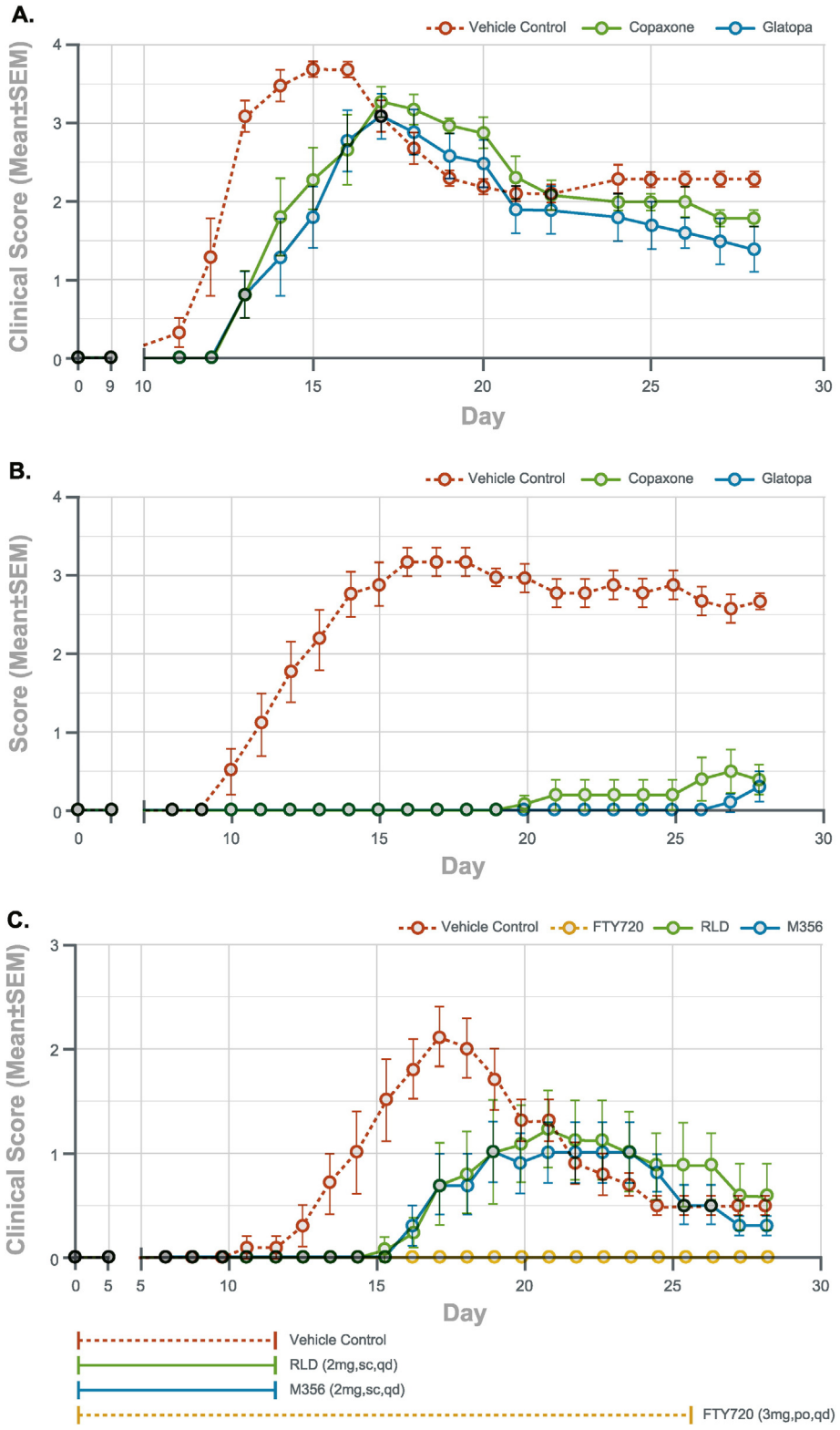


Fig. 10. Glatopa and Copaxone similarly delayed the onset of symptoms compared with controls in experimental autoimmune encephalomyelitis models. (A) Clinical scores: active induction with proteolipid peptide $(PLP)_{139-151}$. (B) Clinical scores: active induction with myelin oligodendrocyte glycoprotein (MOG_{35-55}). (C) Clinical scores: adoptive transfer from $PLP_{139-151}$. FTY720, fingolimod (used as a positive control; inhibited disease when given daily at 3 mg/kg).

sclerosis (MS) in humans and to test the efficacy of potential therapies [4]. EAE models of relapsing–remitting and chronic and progressive forms of MS have been developed and are used to predict potential clinical efficacy of new therapies for MS [26]. In fact, the EAE model is currently used as a release test to confirm the biological activity of Copaxone. EAE was induced with various immunogenic myelin neuroantigens, either directly by immunization with these antigens (i.e., active induction) or passively following transfer of lymphocytes specific to these neuroantigens. Three different mouse EAE models were used to compare the efficacy of Glatopa and Copaxone [27].

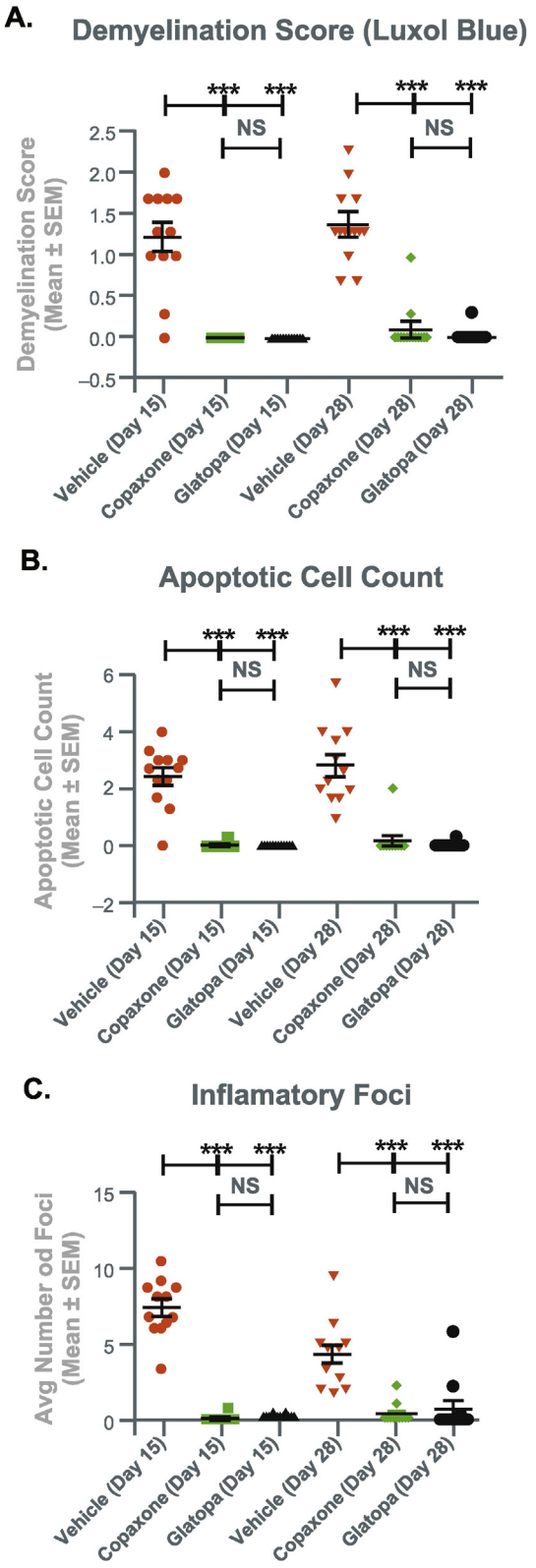


Fig. 11. Histological analysis of myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ active induction experimental autoimmune encephalomyelitis model. A) The extent of demyelination, B) apoptotic cell counts, and C) inflammatory foci. NS, not significant. $^{***}P < 0.001$ vs. controls. One-way ANOVA followed by Tukey's method for multiple comparisons.

All animal experiments were approved and performed under the guidelines of the Institutional Animal Care and Use Committee of Momenta Pharmaceuticals, Inc. (IACUC approval number 05-2011).

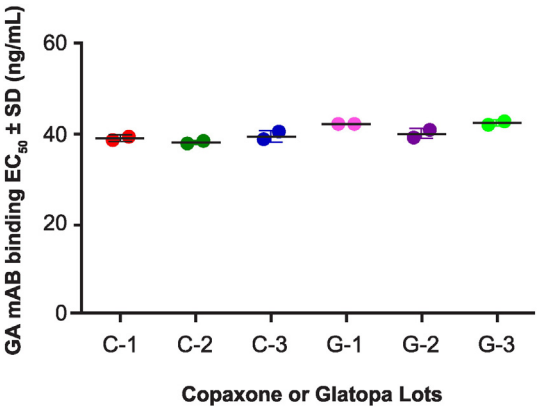


Fig. 12. Sandwich enzyme-linked immunosorbent assay. An immunorecognition assay using a panel of GA-specific monoclonal antibodies to map epitopes showed that the EC_{50} values of three lots each of Glatopa and Copaxone were not significantly different for the monoclonal antibody pair shown (P = not significant). C, Copaxone; G, Glatopa.

In the first model, EAE is induced in SJL/J mice by immunization with proteolipid peptide ($PLP_{139-151}$). The active-induction $PLP_{139-151}$ model was chosen because it is a well-established simulation of relapsing–remitting MS (RRMS) [28,29]. It simulates the various steps of autoimmune antigen recognition and presentation, T-cell activation and polarization, trafficking of autoreactive inflammatory cells, initiation of inflammation in the CNS, and eventual resolution of disease.

In the second model, active induction was initiated by immunization of C57Bl/6 mice with the neuroantigen myelin oligodendrocyte glycoprotein (MOG_{35-55}) [30]. This active induction model is a well-established simulation of primary progressive MS. Similar to the $PLP_{139-151}$ model, MOG_{35-55} induction mimics the various steps of autoimmune response but exhibits more neurodegeneration [26]. It was, therefore, chosen as a confirmatory model to compare the neuroprotective effects of Glatopa and Copaxone.

Finally, in the adoptive transfer model of EAE, SJL/J donor mice were immunized with $PLP_{139-151}$, donor spleens were removed, and splenocytes were isolated for culture in the presence of $PLP_{139-151}$. Cells were then given intravenously to naive recipient SJL/J mice [27]. Glatopa or Copaxone at 2 mg was given daily on days 0 through 9. The adoptive transfer $PLP_{139-151}$ model is another well-established simulation of RRMS that bypasses the T-cell activation process and focuses on more downstream aspects of the disease, such as lymphocyte trafficking, homing to the CNS, and resolution of inflammation. This model was chosen to compare the effects of Glatopa and Copaxone on these aspects of the autoimmune response, using a different, daily therapeutic dosing regimen [29].

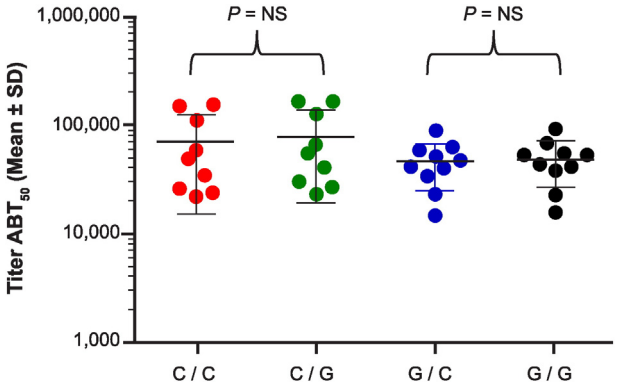


Fig. 13. B-cell biology. No statistically significant differences in the antibody titers obtained in mice immunized with Copaxone or Glatopa independent of the capture antigen. C, Copaxone; G, Glatopa; NS, not significant.

All three EAE models (Fig. 10) demonstrated equivalent efficacy between Glatopa and Copaxone and statistically significant delays in disease onset. In addition, histological examination of the $MOG_{35–55}$ study (Fig. 11) confirmed a strong inhibition of inflammation, as measured by reduced immune cell infiltration, and evidence of neuroprotection, as measured by diminished damage to myelin sheaths and decreases in apoptotic cell counts. Importantly, there was no significant difference between Glatopa and Copaxone in any of the following measured disease parameters: incidence, intensity, mean peak score, and mean day of onset.

To assess the immunogenic potential of GA, in vitro and in vivo assays were employed. Unlike protein therapeutics, GA has secondary but no tertiary structure. Unlike biological therapeutics, GA is also not susceptible to aggregation, which could uncover epitopes that are recognized by the immune system and thereby lead to the formation of antibodies. Nevertheless, GA is considered a therapeutic vaccine, and as such, the activation of the humoral immune system with the resulting development of antibodies to Copaxone following repeated treatment is well known [13,31]. These antibodies are non-neutralizing and do not negatively impact the safety and efficacy of Copaxone; in fact, high titers of antibodies have been correlated with improved clinical response [32]. Differences in immunogenicity were not expected between Glatopa and Copaxone, which had already demonstrated structural equivalence, but two immunological methods were used to specifically confirm this.

The first was an immunorecognition assay using a panel of GA-specific monoclonal antibodies (mAbs) to map epitopes. Epitope-recognition testing using sandwich ELISA with GA-specific mAbs showed that the EC_{50} values of three lots of Glatopa and three lots of Copaxone were not statistically significantly different for the mAb pair shown (Fig. 12). The epitopes recognized by the mAbs were found in the same abundance in both Glatopa and Copaxone. This showed evidence of a similar “immuno-fingerprint” between these two versions of GA, and demonstrated that the amino acid composition and sequences of both drugs are equivalent.

The second test was a formal in vivo demonstration of comparable immunogenic potential of the two GAs and was performed by measuring the temporal readout of antibody response (titer and isotype) in mice and the cross-reactivity of antibodies generated in individual mice. The antibody titers of mice treated with Copaxone or Glatopa were assessed in a crossover design (Fig. 13). Sera samples from mice immunized with Copaxone or Glatopa generated robust antibody titers at day 28 that cross-reacted equally (i.e., there were no statistically significant differences, $P < 0.05$) with both antigens within each individual animal. These studies comprehensively demonstrate that there is no higher risk of immunogenicity with Glatopa than with Copaxone.

4. Conclusions

A rigorous scientific approach enabled determination of equivalence of a generic GA, Glatopa, with the reference listed drug, Copaxone 20 mg. The most challenging aspect of this was the demonstration of active ingredient sameness, which was achieved using a four-point criteria framework. These criteria included the equivalence of starting materials, process signatures, physicochemical properties, and biological and immunological attributes. The selected methods and data presented here represent a small portion of the comprehensive set of physicochemical and biological assays that were conducted. No differences were observed in structure or function between Glatopa and Copaxone, as measured using more than 45 physicochemical methods and more than 15 biological and immunological assays. In addition to these multiple analyses, as part of the approval process under the ANDA pathway, independent testing by FDA laboratories confirmed the equivalence of Glatopa and Copaxone [33].

The approval of Glatopa by the FDA as fully substitutable for Copaxone 20 mg demonstrates that by using advanced analytics paired with extensive process knowledge, equivalence to the brand product for a complex mixture such as GA is achievable. Furthermore, while GA is not a biologic, the strategies employed and lessons learned in its development, much like those used in the development of the generic complex mixture drug enoxaparin [34], have applications in the development of biosimilars (i.e., generic versions of complex biologic drugs).

Acknowledgments

Momenta Pharmaceuticals, Inc. funded the research mentioned in this article. The authors thank Paul Miller of Momenta, who provided writing support.

References

[1] Copaxone (glatiramer acetate injection) for subcutaneous use[package insert] Teva Pharmaceuticals, North Wales, PA, January 2014.
[2] P.H. Lalive, O. Neuhaus, M. Benkhoucha, D. Burger, R. Hohlfeld, S.S. Zamvil, et al., Glatiramer acetate in the treatment of multiple sclerosis: emerging concepts regarding its mechanism of action, 25 2011, pp. 401–414.
[3] M.K. Racke, A.E. Lovett-Racke, N.J. Karandikar, The mechanism of action of glatiramer acetate treatment in multiple sclerosis, Neurology 74 (Suppl. 1) (2010) S25–S30.
[4] J. Hong, N. Li, X. Zhang, B. Zheng, J.Z. Zhang, Induction of CD4 + CD25 + regulatory T cells by copolymer-I through activation of transcription factor Foxp3, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6449–6454.
[5] R. Aharoni, The mechanism of action of glatiramer acetate in multiple sclerosis and beyond, Autoimmun. Rev. (12) (2013) 543–553.
[6] P.L. Vieira, H.C. Heystek, J. Wormmeester, E.A. Wierenga, M.L. Kapsenberg, Glatiramer acetate (copolymer-1, copaxone) promotes Th2 cell development and increased IL-10 production through modulation of dendritic cells, J. Immunol. 170 (2003) 4483–4488.
[7] O. Neuhaus, C. Farina, H. Wekerle, R. Hohlfeld, Mechanisms of action of glatiramer acetate in multiple sclerosis, Neurology 56 (2001) 702–708.
[8] R. Arnon, R. Aharoni, Mechanism of action of glatiramer acetate in multiple sclerosis and its potential for the development of new applications, Proc. Natl. Acad. Sci. U. S. A. 101 (Suppl 2) (2004) 14593–14598.
[9] E. Kimby, Tolerability and safety of rituximab (MabThera), Cancer Treat. Rev. 31 (2005) 456–473.
[10] C. Farina, B.F. Then, H. Albrecht, E. Meinl, A. Yassouridis, O. Neuhaus, et al., Treatment of multiple sclerosis with Copaxone (COP): Elispot assay detects COP-induced interleukin-4 and interferon-gamma response in blood cells, Brain 124 (2001) 705–719.
[11] W.H. Stuart, Clinical management of multiple sclerosis: the treatment paradigm and issues of patient management, J. Manag. Care Pharm. 10 (2004) S19–S25.
[12] R.M. Miller, L.E. Happe, K.L. Meyer, R.J. Spear, Approaches to the management of agents used for the treatment of multiple sclerosis: consensus statements from a panel of U.S. managed care pharmacists and physicians, J. Manag. Care Pharm. 18 (2012) 54–62.
[13] Extavia (Interferon Beta-1b)[package insert] Novartis Pharmaceuticals Corporation, East Hanover, NJ, 2014.
[14] Betaseron (Interferon Beta-1b) for Injection, for Subcutaneous Use[package insert] Bayer HealthCare Pharmaceuticals Inc., Whippany, NJ, January 2014.
[15] Avonex (Interferon Beta-1a) Injection, for Intramuscular Injection[package insert] Biogen Idec Inc., Cambridge, MA, March 2013.
[16] Rebif (Interferon Beta-1a), for Subcutaneous Injection[package insert] EMD Serono Inc., Rockland, MA, April 2014.
[17] Glatopa (Glatiramer Acetate Injection), for Subcutaneous Use[package insert] Sandoz Inc., Princeton, NJ, 2014.
[18] US Food and Drug Administration, Abbreviated New Drug Applications: Generics, US Food and Drug Administration, Silver Spring, Maryland, 2014 Available at: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/AbbreviatedNewDrugApplicationANDAGenerics. Accessed July 16, 2015.
[19] US Food and Drug Administration. Code of Federal Regulations Title 21. 21. Bioavailability and Bioequivalence Requirements. Sec. 320.1 Definitions. Silver Spring, Maryland: US Food and Drug Administration, 2014. CFR320.1. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=320.1. Accessed July 16, 2015.
[20] US Food and Drug Administration. CFR — Code of Federal Regulations Title 21. 2014. Part 320.22 Criteria for Waiver of Evidence of In Vivo Bioavailability or Bioequivalence. 21CFR320.22. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm. Accessed July 16, 2015.
[21] D. Teitelbaum, A. Meshorer, T. Hirshfeld, R. Arnon, M. Sela, Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide, Eur. J. Immunol. 1 (1971) 242–248.
[22] Hsiao TY, Ho MF. Synthesis of glatiramer acetate. US Patent Application Publication. Pub. No.: US 2010/0036092 A1. February 11 2010.
[23] J.M.J. Cowie, V. Arrighi, Polymers: Chemistry and Physics of Modern Materials, 3rd ed. CRC Press, Boca Raton, 2007.
[24] J.S. D'Alessandro, J. Duffner, J. Pradines, Comparative gene expression profiling between a generic (Glatopa™) and brand Copaxone® (glatiramer

acetate injection), Presented at the 2015 AAN Annual Meeting. April 20 2015; Washington, DC, 2015.
[25] R. Aharoni, D. Teitelbaum, M. Sela, R. Arnon, Copolymer 1 induces T cells of the T helper type 2 that crossreact with myelin basic protein and suppress experimental autoimmune encephalomyelitis, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 10821–10826.
[26] R. Gold, C. Linington, H. Lassmann, Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research, Brain 129 (2006) 1953–1971.
[27] C. Honan, T.C. Gangly, I. Fier, G.V. Kaundinya, Comparative efficacy between a generic (M356) and brand Copaxone® (glatiramer acetate injection) in an animal model of multiple sclerosis, Presented at the 2014 Joint ACTRIMS—ECTRIMS Meeting; September 12 2014, Boston, MA, 2014.
[28] B.L. McRae, M.K. Kennedy, L.J. Tan, M.C. Dal Canto, K.S. Picha, S.D. Miller, Induction of active and adoptive relapsing experimental autoimmune encephalomyelitis (EAE) using an encephalitogenic epitope of proteolipid protein, J. Neuroimmunol. 38 (1992) 229–240.
[29] D. Teitelbaum, M. Fridkis-Hareli, R. Arnon, M. Sela, Copolymer 1 inhibits chronic relapsing experimental allergic encephalomyelitis induced by proteolipid protein (PLP) peptides in mice and interferes with PLP-specific T cell responses, J. Neuroimmunol. 64 (1996) 209–217.
[30] A. Ben-Nun, I. Mendel, R. Bakimer, M. Fridkis-Hareli, D. Teitelbaum, R. Arnon, et al., The autoimmune reactivity to myelin oligodendrocyte glycoprotein (MOG) in multiple sclerosis is potentially pathogenic: effect of copolymer 1 on MOG-induced disease, J. Neurol. 243 (1996) S14–S22.
[31] J. Conner, Glatiramer acetate and therapeutic peptide vaccines for multiple sclerosis, J. Autoimmun. Cell Resp. (2014) 1–3.
[32] T. Brenner, R. Arnon, M. Sela, O. Abramsky, Z. Meiner, R. Riven-Kreitman, et al., Humoral and cellular immune responses to Copolymer 1 in multiple sclerosis patients treated with Copaxone, J. Neuroimmunol. 115 (2001) 152–160.
[33] Regulations.gov, Citizen petition denial letter from CDER to Teva Pharmaceuticals; Docket No. FDA-2015-P-1050-012Available at http://www.regulations.gov/#!documentDetail;D=FDA-2015-P-1050-00122015 (Accessed July 16, 2015).
[34] S. Lee, A. Raw, L. Yu, R. Lionberger, N. Ya, D. Verthelyi, et al., Scientific considerations in the review and approval of generic enoxaparin in the United States, Nat. Biotechnol. 31 (2013) 220–226.