Journal of Pharmaceutical Sciences xxx (2016) 1-18

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Review

New and Evolving Techniques for the Characterization of Peptide Therapeutics

Suzanne M. D'Addio [1, *], Jameson R. Bothe [2], Claudia Neri [2], Paul L. Walsh [2], Jingtao Zhang [3], Elizabeth Pierson [2], Yun Mao [2], Marian Gindy [3], Anthony Leone [3], Allen C. Templeton [1]

[1] Pharmaceutical Sciences and Clinical Supply, Merck Research Laboratories, Merck & Co., Inc., 2000 Galloping Hill Rd, Kenilworth, New Jersey 07033
[2] Pharmaceutical Sciences and Clinical Supply, Merck Research Laboratories, Merck & Co., Inc., 126 E. Lincoln Avenue, Rahway, New Jersey 07065
[3] Pharmaceutical Sciences and Clinical Supply, Merck Research Laboratories, Merck & Co., Inc., 770 Sumneytown Pike, West Point, Pennsylvania 19486

ARTICLE INFO

Article history:
Received 15 March 2016
Revised 6 June 2016
Accepted 17 June 2016

Keywords:
peptides
physicochemical properties
chemical stability
physical characterization
stability
chromatography
liquid chromatography
analytical chemistry
formulation

ABSTRACT

Advances in technologies related to the design and manufacture of therapeutic peptides have enabled researchers to overcome the biological and technological challenges that have limited their application in the past. As a result, peptides of increasing complexity have become progressively important against a variety of disease targets. Developing peptide drug products brings with it unique scientific challenges consistent with the unique physicochemical properties of peptide molecules. The identification of the proper characterization tools is required in order to develop peptide formulations with the appropriate stability, manufacturability, and bioperformance characteristics. This knowledge supports the build of critical quality attributes and, ultimately, regulatory specifications. The purpose of this review article is to provide an overview of the techniques that are employed for analytical characterization of peptide drug products. The techniques covered are highlighted in the context of peptide drug product understanding and include chemical and biophysical approaches. Emphasis is placed on summarizing the recent literature experience in the field. Finally, the authors provide regulatory perspective on these characterization approaches and discuss some potential areas for further research in the field.

Introduction

A wave of increasingly complex peptide therapeutics being approved in recent years has highlighted the need for modern analytical approaches to characterize these systems. Peptide therapeutics have the potential to provide patients with novel treatment options in medically diverse areas. With that comes the potential for strong product revenues, with value estimates for the global peptide market at $23.7 billion by 2020.[1] Hence, there is continued interest in further exploring peptides as an effective means for disease treatment in major therapeutic areas, including metabolic disorders, skin infections, and oncology.[1] The number of peptide new molecular entities (NMEs) approved by the Food and Drug Administration (FDA) per decade has been rapidly increasing (Fig. 1),[2] in contrast to the number of all NMEs approved per decade over the same time period.[3] This trend for peptides can be expected to continue, as 17 peptide NMEs have already been approved from 2010 to 2014 and there are estimates of >100 programs in Phase II/III and >150 programs in Preclinical/Phase I development.[4]

The field of peptide characterization is broad, has a rich history, and, in general, is relatively well-understood at this point. However, given the relatively nascent but growing state of peptide characterization in the context pharmaceutical product development, the objective of this review is to elaborate upon the methods and challenges related to analytical characterization of peptide therapeutics. The tools developed for use during drug discovery or for the analytical treatment of peptides as active pharmaceutical ingredients are out of scope of the discussion.

There is much diversity in the size, structure, chemical modifications, and manufacture routes for therapeutic peptides, and there are a variety of ways in which peptides have been distinguished from proteins. In the peer-reviewed literature, this distinction is less significant; however, for regulatory filings of therapeutic molecules, this distinction dictates the regulatory guidance and expectations that apply to a given program. The objectives of pharmaceutical development include the definition of drug products to support clinical studies and registration filings; hence, we

* *Correspondence to:* Suzanne M. D'Addio (Telephone: +1-908-740-4325; Fax: +215-616-1367).
E-mail address: suzanne.daddio2@merck.com (S.M. D'Addio).

http://dx.doi.org/10.1016/j.xphs.2016.06.011
0022-3549/

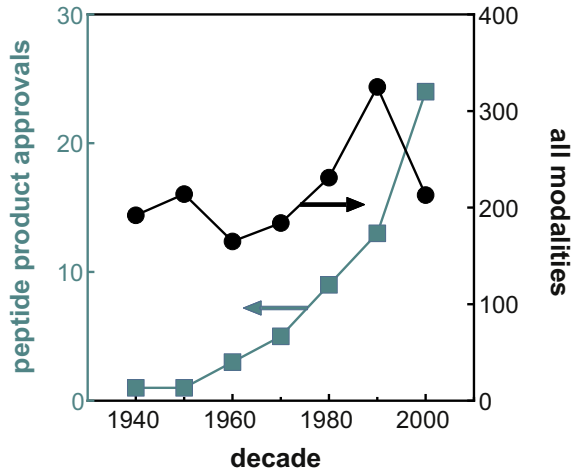

Figure 1. Trends in (■) the number of peptide NMEs approved by the FDA per decade, from 1940 to 2009, excluding generic approvals and line extension products[2] and (●) the total number of NMEs approved by the FDA per decade.[3]

highlight the key differences in existing guidelines for the evaluation of peptides. The European Medicines Agency (EMA) and Japan Pharmaceutical Manufacturers Association consider the method of manufacture or source of raw materials to be the major distinction between proteins and peptides,[5-7] while the FDA currently considers each case individually, with a proposed guidance considering both the method of manufacture and the size of the molecule in delineating peptides and proteins.[8] The context for this review is the development of molecules that are considered peptides by regulatory agencies, including peptide small molecule conjugates, and therefore not developed following guidances for biologics. With this definition, peptide therapeutics represent a middle ground between classical small molecule therapeutics and biologics. The complexity in characterizing peptides results from sitting in this niche: the full set of tools optimized for characterization of large molecules is not 100% value-added, while traditional small molecule assessment toolkits are not capable of fully establishing a fundamental understanding of a peptide in a formulated drug product.

The techniques and studies described in this review are meant to examine contemporary literature and thinking in the field as it pertains to using chemical and biophysical analysis approaches to support fundamental understanding during drug product development and formulation of peptide therapeutics. The analytical characterization of peptide therapeutics also involves bioactivity assays, meant to probe biological features in the context of *in vitro* or cell-derived assays, but this area is left out of scope of this review in favor of a closer examination of techniques that explore physicochemical features of peptide therapeutic systems. As with other modalities, the endgame for peptide product development is to achieve a registered product that serves the needs of patients and medical practitioners. Thus, analytical characterization as it pertains to defining the final product quality and specifications are in scope, along with the advancing state of regulatory oversight in the area. The need for such a review is compelling as there is currently a paucity of review articles that bring together the relevant analytical tools and approaches for the characterization of peptide drug product therapeutics. Furthermore, the application of the analytical techniques discussed herein is relatively nascent and distinct from the application of these techniques in the more familiar space associated with small molecules or biologics. The review is organized to cover chemical analysis approaches, physical analysis approaches, the current state of regulatory guidance in the field, and concludes with a discussion that highlights the gaps in understanding and potential future directions in this evolving field.

Chemical Analysis Techniques for the Characterization of Peptide Therapeutics

During peptide drug product development, the objectives of chemical analysis minimally include confirmation of the peptide identity, assay, tracking the growth of degradation products, and identification of those that grow above the thresholds set in the International Conference on Harmonization (ICH) Q3B guidelines. For decades, chromatographic methods for separation and quantification have been applied to the isolation and purification of peptides of interest from various biological sources[9]; hence, these methods are rather mature and typically serve as a reliable starting point for the resolution of various peptide-related substances in peptide drug products. In recent years, reports on the use of ion mobility spectroscopy (IMS) and mass spectrometry for separation and characterization of peptides have increased, although chromatographic methods continue to be the predominant methods relied upon for routine analysis.

The focus in this section is a deeper dive on methods used for the separation and analysis of a peptide from its related process impurities and degradation products in the drug product, which may be structurally very similar to the parent peptide, and present unique challenges in separation and characterization. First, we review chromatographic methods of separation and analysis. The discussion of advancements in these techniques and aspects unique to peptides is particularly of use to those handling the development of synthetic peptides. Following this, the use of mass spectrometric methods are presented in the context of identifying a peptide therapeutic by molecular weight while also providing information on primary sequence and related degradation products. Finally, we conclude the section by highlighting several practical topics related to the analysis of peptide drug products, including the impact of degradation on physicochemical properties, complications arising from metal contamination, analysis of novel peptide formulations, characterization of PEGylated peptides, and practical aspects of handling peptides in an analytical laboratory.

Separation and Quantification of Peptides and Their Related Substances

Overview of Chromatographic Methods

High performance liquid chromatography (HPLC) is the primary analytical method currently used for separating and quantifying peptides and related degradation products, which is supported by the body of literature that describes the analysis peptides with diverse properties and complexity.[10-12] While therapeutic human pituitary hormones do not fall under the definition of peptides as set out in this article, there is a large body of literature from the 1990s and early 2000s which was critically reviewed by Ribela et al.,[12] which serves as an excellent summary for specific findings related to the application of various chromatography modes to the analysis of peptides. In cases of more challenging mixtures, ultra high performance liquid chromatography (UHPLC) can offer improved resolution and sensitivity compared to HPLC,[13] as was shown by Kelley et al.[14] in the separation and analysis of 23 related species in a solid dosage form of parathyroid hormone. The 3 major modes of HPLC traditionally used in peptide separations utilize differences in either peptide hydrophobicity/hydrophilicity (reverse phase, RP; normal phase or hydrophilic interaction liquid chromatography, HILIC), net charge (ion exchange chromatography, IEC or IEX; capillary electrophoresis, CE), or size (size exclusion chromatography, SEC) (Table 1).[10,15]