FYs 2013-2017 Regulatory Science Report: Complex Mixtures and Peptides

Introduction

Complex drug substances or complex active pharmaceutical ingredients (APIs) include peptides, heterogenous mixtures of small molecules, natural and synthetic polymers, and macromolecular complexes. Their compositional profile may not be easily characterized by current conventional methods and the active components may not be fully defined. Complex drug substances have become more common in approved pharmaceutical products in the past several years. In 2012, the peptide drug market in the United States was less than $11 billion and by 2016 it had grown to more than $18 billion.[1] Complex drug substances are important in many different therapeutic areas and have been used to treat a wide range of conditions, from the use of conjugated estrogens for hormone replacement therapy in post-menopausal women to the use of liraglutide for the treatment of type II diabetes. Complex drug substances are identified in the Generic Drug User Fee Amendments reauthorization (GDUFA II) commitment letter[2] as one of the categories of complex products that are eligible for the enhanced pre-abbreviated new drug application (ANDA) programs for complex products.

Before the Generic Drug User Fee Amendments of 2012 (GDUFA I), research in complex drug substances was limited and focused on a narrow range of products such as synthetic salmon calcitonin[3] and enoxaparin[4]. Research on these products demonstrated that determining API sameness for complex molecules is feasible. In the case of synthetic salmon calcitonin, API sameness and comparability in product and process-related factors were identified as critically important for ensuring the comparability of immunogenicity between the proposed generic and reference listed drug (RLD) products. The approval of salmon calcitonin and enoxaparin became an important foundation for the approval of other generic products containing complex APIs.

Complex active ingredients are diverse and may be mixed with a variety of inactive ingredients in a drug product and thus clear and properly defined API sameness criteria can accelerate the development of generic versions. For peptide drugs, while the active ingredient can be clearly defined and well characterized, characterizing the impurity profile of peptide-related substances and assessing the associated safety risks for things like immunogenicity, is challenging. For complex mixtures, identifying and characterizing active ingredients itself is a daunting task. Food and Drug Administration (FDA) research from the Office of Pharmaceutical Quality (OPQ) and the Office of Generic Drugs (OGD) has been focused on these areas for the past five years. Collectively, our research activities have advanced FDA's understanding of complex drug products containing complex mixtures, helped establish product-specific guidance (PSG) on API sameness, and provided critical information for the regulatory review and decision-making processes.

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[1] Sources: IMS Health National Sales Perspectives[TM]. Year 2012-2016. Extracted August 2017.

[2] https://www.fda.gov/downloads/ForIndustry/UserFees/GenericDrugUserFees/UCM525234.pdf

[3] Lee S, Yu L, Cai B, Johnsons GR, Rosenberg A, Cherney BW, Guo W, Raw A. Scientific Considerations for Generic Synthetic Salmon Calcitonin Nasal Spray Products. AAPS J. 2011;13(1):14-19.

[4] Lee S, Raw A, Yu L, Lionberger R, Ya N, Verthelyi D, Rosenberg A, Kozlowski S, Webber K, Woodcock J. Scientific Considerations in the Review and Approval of Generic Enoxaparin in the United States. Nature Biotechnology, 2013;31(3):220-226.

Accomplishments (2012-2017)

Through our research we have explored and applied modern analytical and quantitative methods to characterize product-specific attributes so that the sameness of the active ingredients can be established between RLDs and proposed generic drugs. During the first 5 years of GDUFA, FDA identified new analytical technologies and used them in the separation and characterization of complex API mixtures and impurities. This research has been performed both internally at the state-of-the-art OPQ laboratories and through external partnerships with academic institutions. For example, ion-mobility spectrometry mass spectrometry (IMS-MS) and complex two-dimensional nuclear magnetic resonance spectroscopy (2-D NMR) techniques, and separation and characterization of complex natural products were used in characterizing the complex API mixtures and providing a basis for demonstrating API sameness.

FDA also conducted research in peptide impurity identification and characterization using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to analyze calcitonin salmon nasal spray products from different manufacturers. FDA also studied immunogenicity potential of these peptide products in vitro using a cell-based assay. Computational algorithms, commonly referred to as big data analysis, based on LC-MS data acquired at FDA allowed FDA to identify key structural fingerprint information of glatiramer acetate, a mixture of synthetic peptides of varying lengths and sequences of four amino acids, creating a foundation for evaluating API sameness and generic drug approval. Internal research led to the approval of generic glatiramer acetate in 2015 and publication of the glatiramer acetate PSG in 2016. Through GDUFA-funded research, FDA published several new PSGs for complex API drugs, and revised many other PSGs to include API sameness. All these achievements were made possible with the GDUFA-funded research support.

Research and Collaborations

Extramural Projects
Integrated Approach to Determine Equivalence in Complex Drug Mixtures
FDA awarded Grant 1U01FD005291 to Ram Sasisekharan at Massachusetts Institute of Technology on September 10, 2014, and the study was completed in August 2017. The grant aimed to characterize the complex drug pentosan polysulfate sodium (PPS) and use the information gathered in the characterization process to build mathematical models. This information can be used to predict the structures of the individual components and their relative abundances in the complex mixture. Researchers identified analytical technologies and implemented them at different levels based on heterogeneity of the APIs to provide orthogonal measurements on overlapping structural attributes of the API between each level; the structural attributes obtained from those measurements were cross validated to make sure the variations in the attributes were captured in the measurements. The data were used to develop a model that describes the varying chemical compositions of the API and evaluate the sufficiency of API characterization and API sameness in composition.

Development of an Integrated Mathematical Model for Comparative Characterization of Complex Molecules
FDA awarded Grant 1U01FD005285 to David Volkin at the University of Kansas on September 10, 2014, and the study was completed in August 2017. The goal of this grant was to build a mathematical model using data collected in the characterization of complex molecules to assess the similarity and determine if the data were sufficient for API sameness evaluation. The active ingredient and its degradation products were studied with a variety of physicochemical and biological assays to characterize the critical

quality attributes. All the data collected were used to develop a mathematical model which can be used to assess API sameness of generic drug applications.[5,6,7]

Statistical Methodology for Characterization of Macromolecular Similarity

FDA awarded Grant 1U01FD005288 to John Cort at Battelle Pacific Northwest National Laboratories on September 10, 2014, and the study was completed in August 2017. The goal of this project was to develop a statistical methodology to assess macromolecular similarity of pentosan polysulfate (PPS). In this project, IMS-MS and 2D-NMR data were collected on PPS and these data were used to build PPS signatures. Similarity between two sets of data were measured and variability was captured with a subset of features so that sufficiency may be established through a similarity metric. The data collected were used to develop a macromolecular similarity model which can be used to assess similarity between different samples.

Mass Spectrometry Profiling of Pentosan Polysulfate in Urine

FDA awarded Contract HHSF223201610114C to John Cort at Battelle Pacific Northwest National Laboratories on September 22, 2016, and the project is ongoing. The goal of this project is to use MS to profile PPS and its metabolites in urine with the final goal of assisting with the development of a bioanalytical method to establish bioequivalence for PPS as an alternative to clinical endpoint bioequivalence studies. In this project, it was found that reverse phase ion-pair chromatography can separate PPS from salts, metabolites, and other small molecules in urine prior to analysis with a mass spectrometer. High resolution and accurate MS can help the identification and characterization of PPS components. Preliminary data show that urine samples from patients taking PPS have a different profile compared to urine samples spiked with PPS; indicating PPS in patient urine is different from the original PPS.

Internal Projects
Characterization of Glatiramer Acetate

Glatiramer acetate is a mixture of synthetic peptides containing four amino acids, glutamic acid, alanine, tyrosine, and lysine, with an average molecular weight of 5,000- 9,000 Daltons. FDA labs used the brand-name product, COPAXONE®, and a commercially available comparator, Copolymer-1, to develop a set of characterization methods for comparison and characterization of glatiramoids. These orthogonal approaches include asymmetric field flow fractionation coupled with multi-angle light scattering (AFFF-MALS), NMR and LC-MS. AFFF-MALS was used to calculate the average molecular weight, M_w, polydispersity, and provided molecular weight distribution of the polymers. NMR was used to evaluate the amino acid composition and LC-MS was used to separate and identify the peptide fragments generated from enzymatic degradation. Principal component analysis (PCA) was applied to LC-MS results to identify differences between lots of COPAXONE® and comparator lots. These methods, combined, constitute an analytical approach to characterize and compare glatiramoids. These methods have been successfully used in the characterization and evaluation of different generic glatiramer acetate applications, and led to the approval of the first generic glatiramer acetate product in 2015.

[5] Kleindl PA, Xiong J, Hewarathna A, Mozziconacci O, Nariya MK, Fisher AC, Deeds EJ, Joshi SB, Middaugh CR, Schöneich C, Volkin DB, Forrest ML. The Botanical Drug Substance Crofelemer as a Model System for Comparative Characterization of Complex Mixture Drugs. J Pharm Sci. 2017;106(11):3242-3256.

[6] Hewarathna A, Mozziconacci O, Nariya MK, Kleindl PA, Xiong J, Fisher AC, Joshi SB, Middaugh CR, Forrest ML, Volkin DB, Deeds EJ, Schöneich C. Chemical Stability of the Botanical Drug Substance Crofelemer: A Model System for Comparative Characterization of Complex Mixture Drugs. J Pharm Sci. 2017;106(11):3257-3269.

[7] Nariya MK, Kim JH, Xiong J, Kleindl PA, Hewarathna A, Fisher AC, Joshi SB, Schöneich C, Forrest ML, Middaugh CR, Volkin DB. Deeds EJ, Comparative Characterization of Crofelemer Samples Using Data Mining and Machine Learning Approaches with Analytical Stability Data Sets. J Pharm Sci. 2017;106(11):3270-3279.

Analytical Method Development for the Characterization of Conjugated Estrogens
Conjugated estrogens are a mixture of conjugated steroids made from pregnant mares' urine. It is used primarily for estrogen replacement therapy to treat menopausal symptoms and to prevent post-menopausal osteoporosis. The complexity of the chemical composition has been a barrier to the introduction of generics. For this internal project, the FDA labs first developed a robust LC-MS method to characterize and quantify the steroidal components of the conjugated estrogen from the commercial product, PREMARIN®, then the method was used to profile 23 lots of the brand-name product and identified 60 steroidal conjugates which consistently present at average levels above 0.1%. The results of this collaboration have been published[8] and became the foundation of Agency's PSG documents on conjugated estrogens. It has also been used as an analytical tool to evaluate the API sameness of potential generic conjugated estrogens.

Characterization of Impurities in Peptides
Comprehensive characterization of a peptide API is important in ensuring the API sameness for generic peptide drug development. The presence of impurities may lead to altered efficacy and/or safety, including increased immunogenicity risk. The collaborations were aimed to develop a sensitive and effective approach to profile impurities in peptide drugs. In an initial study, a data-dependent LC-MS/MS approach was developed to screen low level peptide impurities. This methodology was then applied to the impurity profiling of calcitonin salmon nasal spray, a polypeptide drug for the treatment of osteoporosis. Five marketed calcitonin salmon products were evaluated, and over 120 peptide impurities were identified. Each impurity and its monoisotopic mass and charge states were confirmed by extracted ion chromatography and manual examination of the mass spectra. Differences were observed among different products (Fig. 1, peptide impurities of >0.1% were selected to display differences in samples).
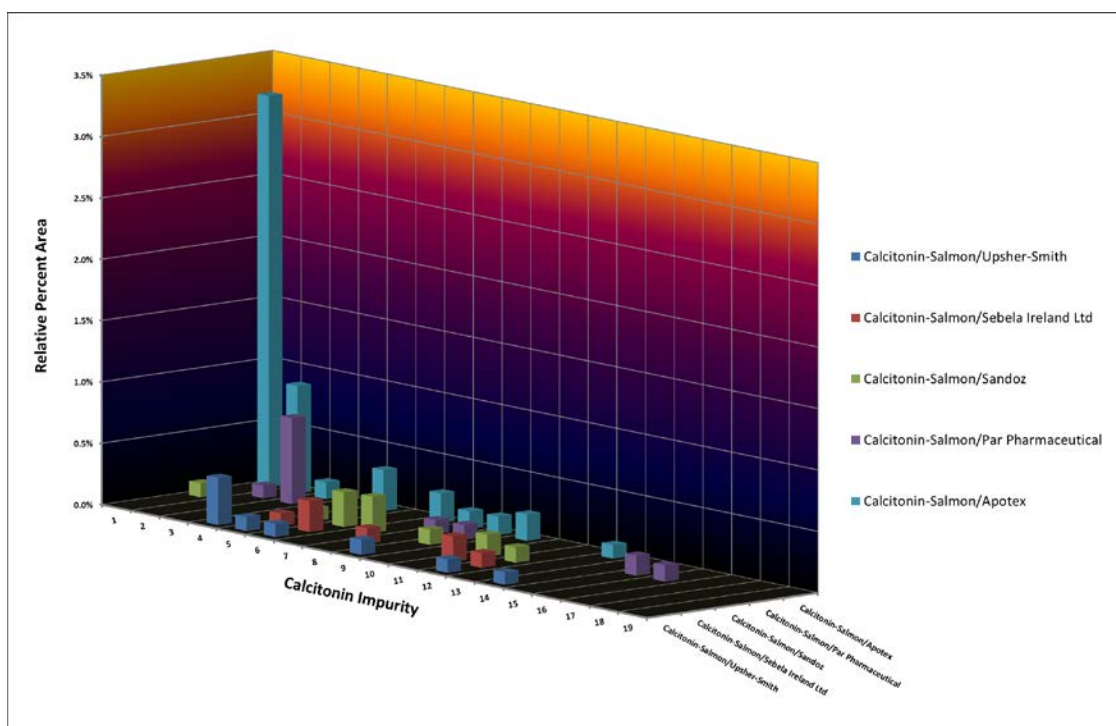

Fig. 1: Differences in Peptide Impurities in Calcitonin Salmon Nasal Spray Products

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[8] Levy MJ, Boyne MTII, Rogstad S, Skanchy DJ, Jiang X, Geerlof-Vidavsky I. Marketplace Analysis of Conjugated Estrogens: Determining the Consistently Present Steroidal Content with LC-MS. AAPS J. 2015;17(6):1438-1445.

This approach allows for screening of those impurities that can be separated from the API peak and observed in total ion chromatography. It also allows for screening of impurities that co-eluted with the API peaks, and those that can be separated from the API peak but cannot be observed as individual peak in the total ion chromatography. This data-dependent LC-MS/MS scan greatly facilitates rapid screening of impurities in peptide drugs and provides an efficient approach for peptide drug quality control and generic drug development. Combination of LC-MS and LC-MS/MS methods provides a powerful tool in analyzing complex peptide mixtures, including mixtures purified from natural sources.

Characterization of Host-cell Protein Impurities in Peptide Products of rDNA Origin
The goal of this ongoing collaboration with OPQ is to develop an MS-based characterization method to identify and characterize host-cell protein (HCP) impurities in peptide drug products of rDNA origin. Literature shows that even highly purified peptide products prepared from recombinant DNA technology contain some HCPs as impurities and different host cell systems used in the manufacturing process would generate different HCPs. The potential immunogenicity risk of the HCPs for brand-name products can be evaluated in clinical studies, but clinical evaluation of immunogenicity risk is usually not part of the ANDA pathway for generic drugs. The Agency does not believe that current technology can provide sufficient information to address potential immunogenicity risk for products produced using recombinant technology without also considering clinical data. FDA is also working to develop new technologies to address this problem. MS can provide very sensitive detection and characterizations of peptides and proteins. Characterization of HCPs with MS will help us understand the HCP impurity level, composition, and impurity control of HCPs in the corresponding products. This will open the possibility of accepting ANDAs for peptide drugs produced by recombinant technology in the future.

Assessment of Innate Immune Response Modulating Impurities in Peptide Drugs
Immune response to peptide drugs can compromise the safety and efficacy of the products. Peptide-related impurities in synthetic peptides, HCPs and other impurities in recombinant peptides might be present in peptide drugs which can activate the innate immune system and enhance product immunogenicity. The Office of Biotechnology Products lab recently described a new approach to screen products for the presence of broad spectrum known or unknown innate immune response modulating impurities by using multiple myeloid cell lines. In this collaboration, the lab will test calcitonin salmon nasal sprays manufactured by different companies for their ability to activate innate immune response using the cell based assays. These results will be used to look for a correlation between the impurity profile of the product and the risk of immunogenicity. The benefit of this research to generic drug development is that when immunogenicity risks are identified in product development or review there can be an alternative to clinical studies to address the immunogenicity risk.

Solid State Characterization of Polymeric Drugs
Polymeric drugs such as sevelamer carbonate, patiromer, and colesevelam hydrochloride have been used in the treatment of various diseases. The manufacturing processes of polymeric drugs are complex and involve polymerization of different components. To facilitate the development of generic versions of these drugs, OGD and OPQ worked to characterize these complex polymeric drugs and develop criteria for active ingredient sameness evaluations. Solid state carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR) was used as a powerful tool in studying the composition of the polymeric drugs. Fourier transform infrared spectroscopy, thermogravimetric analysis and differential scanning calorimetrywere used to further analyze the physicochemical properties of these drugs. This project led to a better understanding of the critical quality attributes of the polymeric APIs and revision of several PSGs for polymeric drugs. It also allowed for the approval of the first generic sevelamer carbonate product in 2017.

Key Outcomes

The efforts of Agency research and external collaboration collectively contributed to the understanding of generic drug development for drug products with complex drug substances. During the first five years of GDUFA, the Agency published 12 PSGs for those drug products as part of the effort to establish clear, efficient and consistent regulatory standards for generic products containing complex APIs. These PSGs contributed directly to the development and approval of multiple generic drugs containing complex APIs, including three first generic approvals: glatiramer acetate for injection, sevelamer carbonate tablet, and sevelamer carbonate powder for suspension.

The Agency also published a draft general guidance on ANDA submission of certain highly purified synthetic peptide drug products referencing products of rDNA origin.[9] This guidance provides recommendations for generic competition in a class of reference products where it was previously not generally recommended to use the ANDA submission process.

GDUFA regulatory research projects provided a better understanding on the nature and the complexities of the drug products with complex APIs. Results and insights gained from the research help the development of the PSGs, which directly support the review and approval of the generic versions of these complex products. Research in this area also led to the development of novel analytical technologies and characterization methods and further advanced the science through publications and presentations.

Future Directions

Significant progress has been made in our understanding and development of complex API and peptide products. However, further regulatory research efforts are needed. Research in botanical drugs, which are inherently complex, are in the early stages, and newer brand-name complex API products, such as oligonucleotide drug products, are being approved more frequently. Future research is needed for the development of characterization methods and API sameness standards on natural-sourced complex drug substances; development of sensitive MS-based method to support bioequivalence demonstration in place of traditional clinical endpoint approach on drugs with complex API mixtures; continued development of analytical methods for polymeric drugs; development of analytical methods to characterize complex excipients and their interactions with drug substances; and evaluations on host-cell protein impurities for peptide drug products of rDNA origin and non-clinical immunogenicity assays for generic drug products. These unmet regulatory needs will be a focus in GDUFA II that was reauthorized in 2017 for additional 5 years from 2017-2022. Scientific advances in these areas will help the development of the generic complex drugs which would otherwise be impossible. These future regulatory research priorities will contribute significantly to the ability of the American public to access generic version of these complex products.

Outcomes

Product-Specific Guidances
- Revision of Draft product-specific guidance on Colesevelam Hydrochloride Tablet (Jul 2008; revised Dec 2009; Nov 2010; Jun 2011; Nov 2013; Sep 2015)
  https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm083337.pdf
- Revision of Draft product-specific guidance on Colesevelam Hydrochloride Powder for Suspension (Aug 2010; revised Jun 2011; Mar 2012; Jun 2013; Jan 2016)

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[9] https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365

https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM224204.pdf

- Posting of Draft product-specific guidance on Conjugated Estrogen Tablet (Dec 2014)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM428204.pdf
- Posting of Draft product-specific guidance on Ethiodized Oil Injectable (Dec 2016)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM533302.pdf
- Posting of Draft product-specific guidance on Glatiramer Acetate Injection (Apr 2016)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM495029.pdf
- Revision of Draft product-specific guidance on Omega-3 Carboxylic Acids Capsule (Jan 2016; revised Dec 2016)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM481945.pdf
- Posting of Draft product-specific guidance on Omega-3-acid Ethyl Ester Type A Capsule (Dec 2016)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM533405.pdf
- Revision of Draft product-specific guidance on Omega-3-acid Ethyl Ester Capsule (Sep 2012; revised Oct 2016; Dec 2016)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM320011.pdf
- Revision of Draft product-specific guidance on Pentosan Polysulfate Sodium Capsule (Sep 2012; revised Jul 2014)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM320020.pdf
- Revision of Draft product-specific guidance on Sevelamer Carbonate Powder for Suspension (Jun 2011; revised Dec 2014)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM261528.pdf
- Revision of Draft product-specific guidance on Sevelamer Hydrochloride Tablet (Sep 2008; revised Jul 2009; May 2010; Aug 2010; Aug 2011; Dec 2014)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm089621.pdf
- Revision of Draft product-specific guidance on Sevelamer Carbonate Tablet (Sep 2008; revised May 2010; Aug 2010; Jun 2011; Sep 2015)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm089620.pdf

General Guidances

Draft Guidance for Industry: ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin. (Oct 2017)
https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM578365.pdf

Publications

- Levy M, Boyne MII, Rogstad S, Skanchy D, Jiang X, Geerlof-Vidavsky I. Marketplace Analysis of Conjugated Estrogens: Determining the Consistently Present Steroidal Content with LC-MS. AAPS J. 2015;17(6):1438-1445.

- Rogstad S, Pang E, Sommers C, Hu M, Jiang X, Keire DA, Boyne M. Modern Analytics for Synthetically Derived Complex Drug Substances: NMR, AFFF-MALS, and MS Tests for Glatiramer Acetate. Anal Bioanal Chem. 2015;407:8647-8659.
- Zeng K, Geerlof-Vidavisky I, Gucinski A, Jiang X, Boyne MII. Liquid Chromatography-High Resolution Mass Spectrometry for Peptide Drug Quality Control. AAPS J. 2015;17(3):643-651.

Presentations
- Kedia K, Zheng X, Zhou M, Ansong C, Baker ES, Cort JR. Mass Spectrometry Profiling of Pentosane Polysulfate Sodium (PPS). 65th ASMS Conference on Mass Spectrometry and Allied Topics, June 4-8, 2017, Indianapolis, IN.
- Yang J. Rapid Screening of Peptide Impurities in Calcitonin-Salmon Nasal Spray Using Data-Dependent LC-MS-MS and Data-Independent LC-MS. 65th ASMS Conference on Mass Spectrometry and Allied Topics, June 4-8, 2017, Indianapolis, IN.
- Zhang D, Jhon Y, Ye Y, Berendt R, Cai B, Jiang X. Development of Product-Specific Guidance for Polymeric Drugs: A Case Study on Sevelamer and Colesevelam. American Association of Pharmaceutical Scientists (AAPS) Annual Meeting, November 13, 2016, Denver, CO.
- Zhang D. Scientific Considerations in Submitting Synthetic Peptide Drug Products as ANDAs Referencing Peptide Drug products of rDNA origin. 3rd Synthetic Therapeutic Peptide Workshop, November 14-15, 2016, Rockville, MD.