Considerations in Developing Generic Peptide and Oligonucleotide Drug Products

USP Workshop on Peptide and Oligonucleotide Therapeutics

Regulations, Standards and Quality

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Outline

- Introduction: Active Pharmaceutical Ingredient (API) Sameness for an ANDA*
- Regulatory Considerations on Generic Synthetic Peptides
- Preliminary Thinking on Generic Synthetic Oligonucleotides

*ANDA: Abbreviated New Drug Application

Generic Drugs and API Sameness

A generic drug must be therapeutically equivalent* to the reference listed drug (RLD)

- **Pharmaceutical equivalent**
- Bioequivalent
- Adequately labeled
- Manufactured in compliance with cGMP regulations

*For definition, see 21 CFR 314.3(b)

cGMP: current Good Manufacturing Practice

Generic Drugs and API Sameness

To be Pharmaceutical Equivalent (PE), a drug product:

- **Contains same API as the RLD**
- Uses same dosage form and route of administration
- Is identical in strength or concentration
- Meets the same compendial standards for strength, quality, purity and identity

<u>API sameness is a requirement for generic drugs</u>

Active Ingredient Sameness

- Active ingredient defined in 21 CFR 314.3(b)
- ANDA required to contain information to show that the active ingredient is the same as the RLD
  - Section 505(j)(2)(A)(ii) of the Federal Food, Drug, and Cosmetic Act
  - 21 CFR 314.94(a)(5)

Generic Drugs and Bioequivalence

Bioequivalence (BE) is defined as:

- The absence of a significant difference in the rate and extent of absorption compared to the reference drug when administered at the same molar dose of the therapeutic ingredient under similar experimental conditions
  - Most peptide drugs and synthetic oligonucleotide drugs are parenteral solutions for injection, BE may be considered self-evident* and can be waived per 21 CFR 320.22(b)(1)

505(j)(8)(B) of FD&C Act; 21 CFR 320.22(b)

* Generic product should contain same active ingredient and inactive ingredient in the same concentration as the reference product

Generic Peptides and Oligonucleotides

- Focus of the talk:
  - API sameness evaluation
  - Impurity profile assessment (including immunogenicity consideration)

Peptide Drugs

- Peptide: Any alpha amino acid polymer with a defined sequence that is 40 amino acids or fewer in size*;
- Broad interest in developing generic synthetic peptide drugs:
  - Technical development
    - Solid phase peptide synthesis
    - High resolution, sensitive analytical techniques
  - Large commercial market in the United States
    - Liraglutide: $4.8 billions USD in 2018#
    - Teriparatide: $1.0 billion USD in 2018#
  - Evolving regulatory considerations

* FDA Guidance for Industry: *New and Revised Draft Q&As on Biosimilar Development and the BPCI Act (Revision 2)*, December 2018, pp 13-14. # Source: IQVIA, National Sales Perspectives™. Extracted September 2019.

Synthetic Peptides – Regulatory Considerations

- NO ICH* guidelines on peptide quality
- Draft guidance for industry: *ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin* (Oct 2017)
  - Guidance covers five peptide drugs: teriparatide, glucagon, nesiritide, liraglutide and teduglutide;
  - The general characterization principles and strategies in this guidance may be considered when developing other synthetic generic peptide drugs.

*ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Characterization of Peptides

- Primary sequence, amino acid composition
- Optical rotation, other physicochemical properties
- Secondary structure
- Oligomer/Aggregation states
- Biological activities (by in vitro or animal studies)
- Impurities (peptide-related impurities and other impurities)

Use orthogonal analytical methods

Calcitonin-Salmon Impurity Analysis

- Calcitonin-Salmon: a 32 amino acid peptide hormone for postmenopausal osteoporosis;
- Several Calcitonin-Salmon nasal spray products have been approved (rDNA and synthetic);
- FDA Lab applied a data-dependent acquisition (DDA) LC-MS-MS and data-independent acquisition (DIA) LC-MS^E approach to analyze peptide impurities in Calcitonin-Salmon nasal spray.

Rapid Screening of Peptide Impurities in Calcitonin-Salmon Nasal Spray Using Data-Dependent LC-MS-MS And Data-Independent LC-MS^E, Yang, J., et. al., ASMS Poster, 2017.

Calcitonin-Salmon Impurity Analysis

- Instruments: UHPLC-MS (Thermo Q Exactive Orbitrap and Waters Synapt G2Si mass Spectrometers);
- To identify peptide impurities in all three groups:
  - Impurities observed in total ion chromatogram (TIC);
  - Impurities co-eluting with the API or eluted at its peak tail (challenging for manual screening);
  - Impurities buried under the TIC baseline.

Calcitonin Salmon Impurity Analysis

DIA LC-MS^E approach

Table 1. List of selected peptide impurities observed using DIA approach

Impurity	Rt (min)	M.W. (mono)	Δm/z	% ADC
1	5.8	2195.396	-1234.7	0.01
2		2213.408	-1216.7	0.08
3	21.3	3503.072	73.0	0.02
4		3333.996	-96.1	0.13
5	26.1	3412.084	-18.0	0.01
6	28.7	3412.032	-18.0	0.14
7		3315.960	-114.1	6.45
8		3297.972	-132.1	0.41
9		3477.048	47.0	0.01
10		3572.900	142.8	0.15
11		3214.884	-215.2	0.34
12	30.0	3468.080	38.0	2.18
13		3532.128	102.1	0.02
14		3232.920	-197.2	0.03
15		3501.096	71.0	0.02
16		3451.980	21.9	1.65
17	30.4	3430.072	0.0	0.78
18		2912.788	-517.3	0.01
19		3088.856	-341.2	0.09
20		3316.968	-113.1	0.05
21	31.5	2715.680	-714.4	0.04
22		3431.125	1.1	0.01
23		2930.784	-499.3	0.02
24		3316.968	-113.1	0.05
25		1691.986	-1738.1	0.13
26		3430.072	0.0	0.78
27		3453.000	22.9	0.01
28	32.5	3450.495	20.4	0.73

DDA LC-MS-MS approach

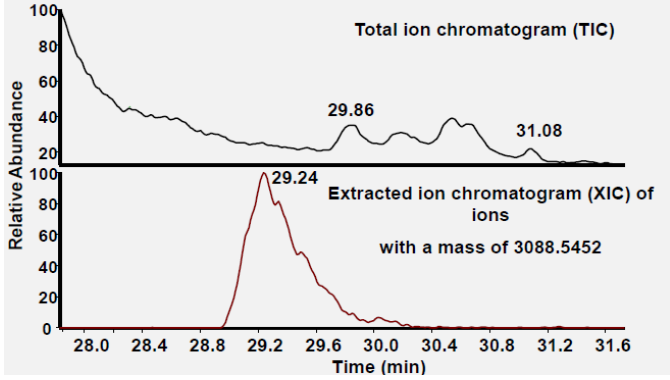
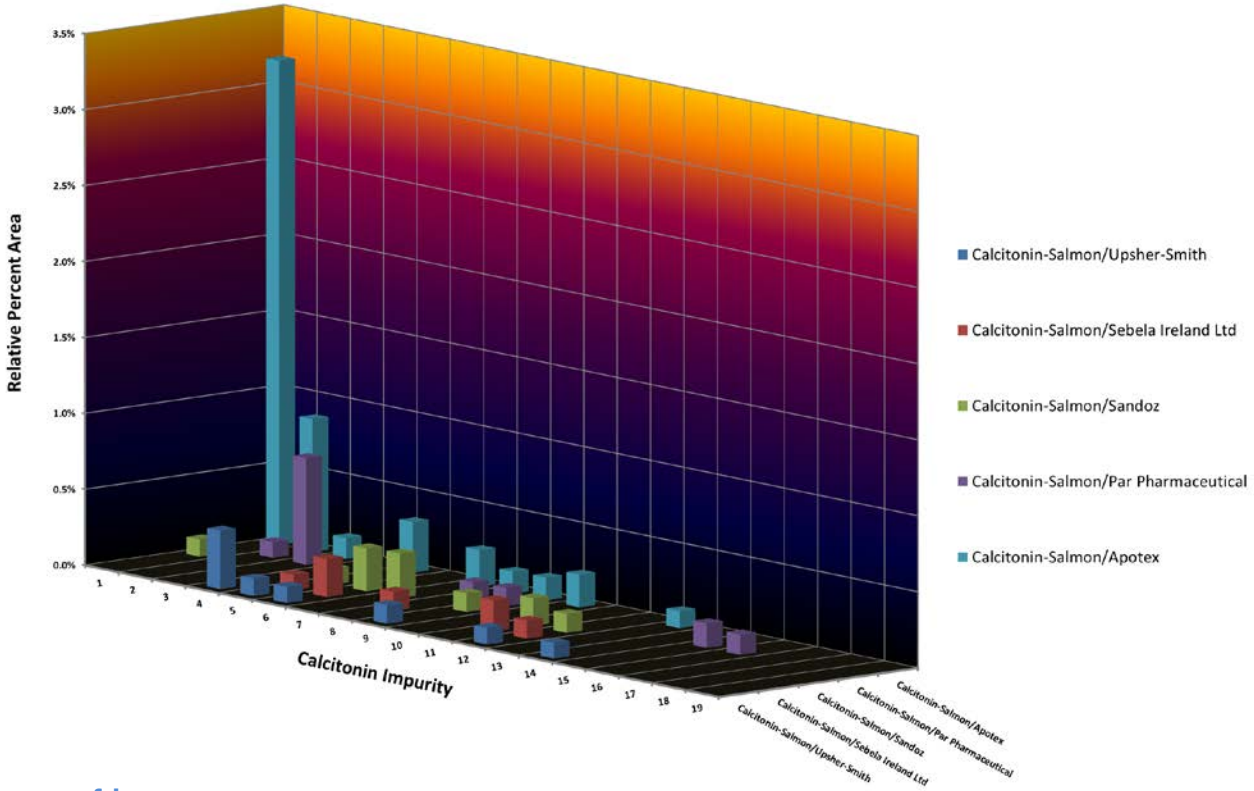


Table 2. List of selected peptide impurities observed in TIC

Index	TIC Peak Retention Time (min)	Monoisotopic Mass	Detected by DDA	Area %
1	5.77	2213.1206	Yes	0.06
2	18.49	1321.6378	No	0.009
3	19.44	1578.7394	No	0.005
4	20.6	3447.7259	Yes	1.17
5		1234.6048	Yes	0.05
6		3333.6461	Yes	0.02
7	24.32	1828.8825	No	0.02
8	24.99	3412.6908	Yes	0.23
9	29.86	2930.4755	Yes	0.04
10	30.4	2715.3867	Yes	0.02
11	30.54	2070.0608	Yes	0.12
12	31.08	1691.8229	Yes	0.06
13	32.71	3471.7261	Yes	0.17
14	35.84	3428.6858	Yes	0.08
15	37.99	3471.7261	Yes	0.02
16	57.07	Only Singly-charged ions observed		
17	58.8	Only Singly-charged ions observed		

API Rt = 28.7 min

Calcitonin-Salmon Impurity Analysis



- 13 nasal spray drug products analyzed;
- Over 100 peptide impurities detected by LC/MS;
- 4 were above 0.5%;
- 16 were above 0.1%.

Peptide Impurities: Comparative Evaluation

To ensure impurities in the proposed generic peptide drug will not alter the safety (including the immunogenicity) and efficacy compared to the RLD product

- In common peptide impurities: generally not higher than those found in the RLD;
- New peptide impurities: need to identify and characterize if above certain threshold;
- To assess immunogenicity risk with in silico, in vitro methods:
  - Individual new peptide impurity: in silico T-cell epitopes, other in vitro assays
  - Generic peptide product: innate immune system

Synthetic Oligonucleotide Drugs

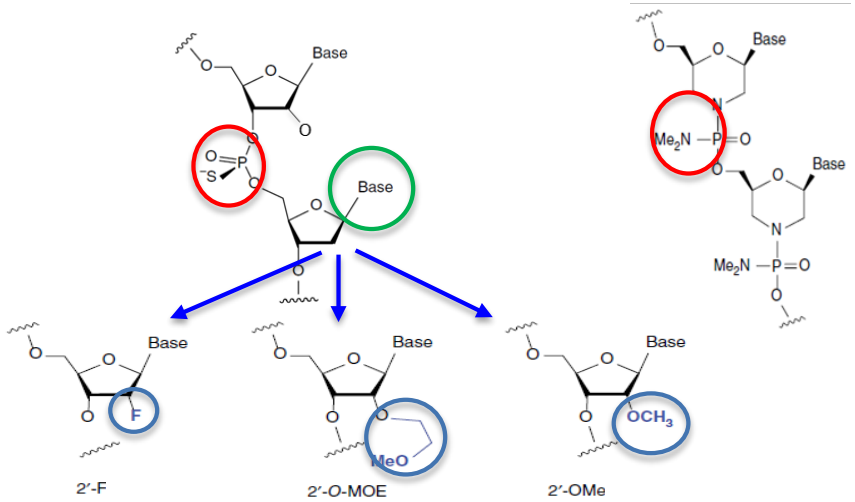
- Synthetic Oligonucleotides (ONs) are chemically synthesized, short nucleic acid chains that can act in a sequence specific manner to control gene expression;
- Increased interest in exploring synthetic ON drugs:
  - Better understanding of biology:
    - could modulate nearly any genetic targets in human genome;
    - could alter target protein production or functions which may have broad therapeutic indications (genetic diseases, cancers, immune system disorders)
  - Improved chemistries: solid phase synthesis
  - Better delivery system
  - Clinical success and regulatory approval of ON drugs

Synthetic Oligonucleotide Drugs

- Antisense oligonucleotides (ASO) are synthetic oligonucleotides, generally 12-30 nucleotides in length that are designed to bind to RNA by Watson-Crick base pairing rules;
- For this talk, we will focus on ASO, and sometimes use synthetic ON and ASO interchangeably;
- Currently approved ASOs:
  - Fomivirsen and Mipomersen (both withdrawn)
  - Eteplirsen, Nusinersen and Inotersen
- Active on-going research will lead to approval of more ASOs.

Synthetic Oligonucleotide Drugs

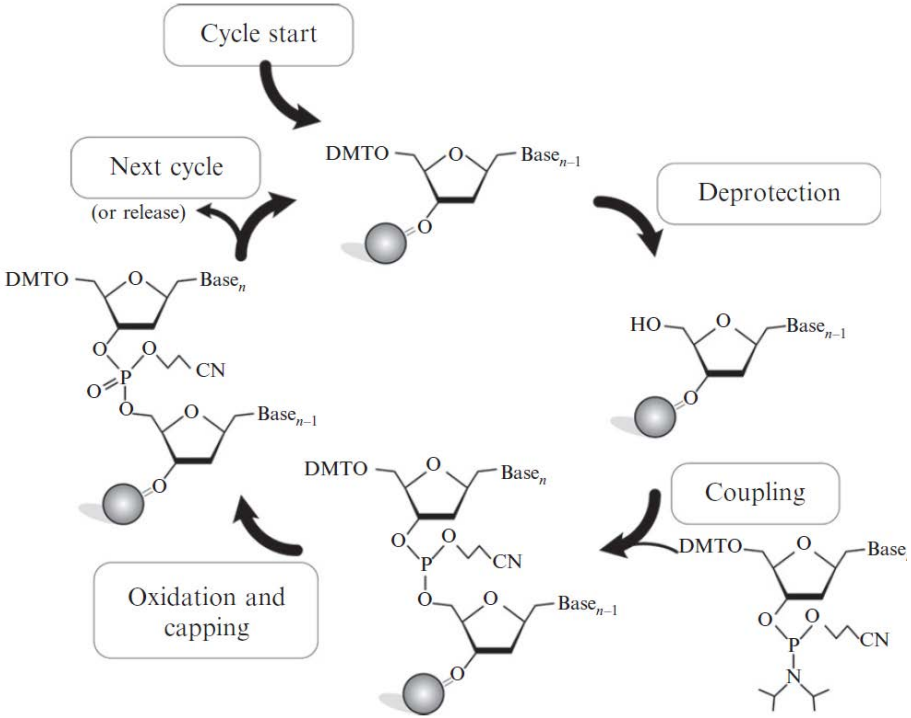
- Common Structural Modifications
  - Backbone
    - Phosphorothioate (PS)
    - Phosphoradiamidate morpholino
  - Base
    - Methylation: ^{Me}C and ^{Me}U
  - Sugar
    - 2'-Substitution (Halides, OMe, O-methoxy-ethyl (MOE)



An ASO may have multiple types of modifications

Oligonucleotide Synthesis

- Diverse solid phase synthesis:
  - First coupling to solid support
  - Each cycle generally includes deprotection, coupling, oxidation/amidation/surfurization, capping
  - Releasing from solid support, final deprotection
- Purification:
  - HPLC (reverse phase; ion exchange)
  - Polyacrylamide gel electrophoresis (PAGE)

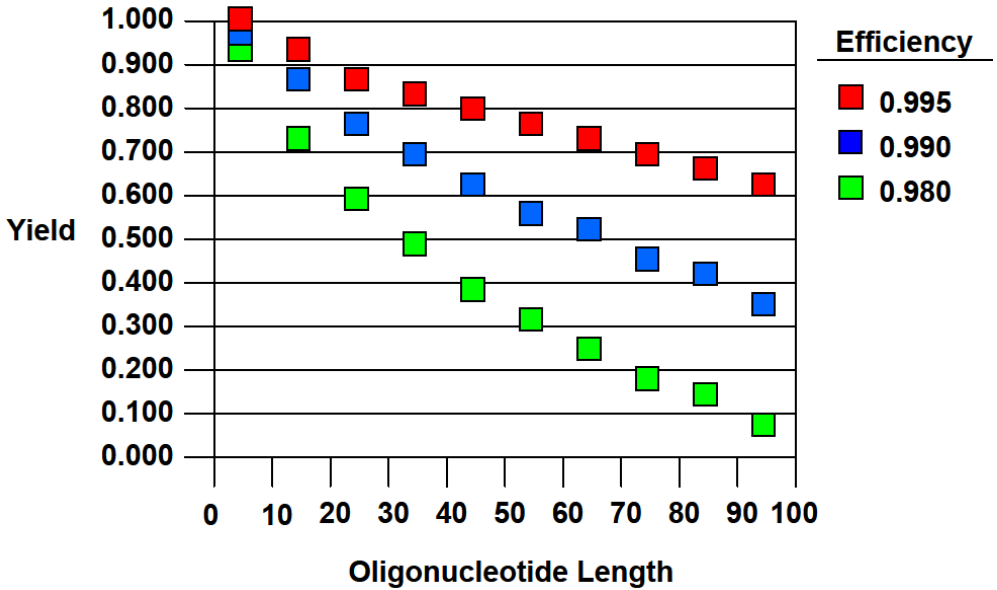


Oligonucleotide synthesis from phosphoramidites. Synthesis proceeds in a 3' →5' direction

Impurities in Oligonucleotides

- Diverse impurity profile:
  - From starting material
  - Incomplete reactions in each cycle (deprotection, coupling, capping, etc.)
  - Side reactions (Abasic ASO)
  - Degradation (monophosphate in phosphorothioate ASO)
- Impurity control is critical for generic synthetic ASO development

Relationship between average coupling efficiency and synthesis yield over the length of the oligonucleotide*



*From Integrated DNA Technologies (2011) Chemical synthesis of oligonucleotides. [Online] Coralville, Integrated DNA Technologies. Available at pages/docs/technical-reports/chemical-synthesis-of-oligonucleotides.pdf

Regulatory Challenges for Generic ASO

- Neither FDA nor European Medicines Agency (EMA) has published any official documentation on the expectation on quality control of ASOs;
- While new ASO approval needs clinical efficacy and safety studies, generic ASO drug relies on the approved RLD for safety and efficacy:
  - Demonstrating API sameness is critical in the approval of any generic ASO drugs

Characterization of ASO

- Structure confirmation
  - Primary sequence; composition, backbone linkage, melting temp, etc.
- Physicochemical properties
  - Molecular weight, spectroscopic data, etc.
- Comparative impurities analysis (product-related impurities and other impurities)
  - Immunogenicity
  - Toxicity

Use orthogonal analytical methods

Comparison of Impurity Types

Peptide-related impurities

- Insertion
- Deletion
- D-Amino acid
- Acylation
- Oxidation
- Deamidation
- Glycosylation

ASO-related impurities

- n+1, n+2,…
- n-1, n-2,…
- Phosphate di-esters in PS-ASOs
- Missing multiple terminal nucleotides
- Base residue change
- Abasic site (loss of base)
- Change in sugar moiety
- Cross-linked impurities (high MW)

Comparison of Impurity Features

Peptide-related impurities

- Often exist in a single, optically pure API
- Oxidation, acylation and deamidation happen on certain amino acids
- 20+ unique amino acids
- UPLC-MS/MS method can provide highly sensitive method for impurity identification/characterization

ASO-related impurities

- Exist in a API which is often a mixture of huge number of diastereomers (2^{n-1})
- Can happen in any nucleotide unit and/or backbone linkage
- Very limited number of bases (A, C, G, T, U), resulting in many repeating nucleotide unit in an ASO
- Currently, very challenging (if not impossible) to identify/characterize a single product-related impurity among all the impurities

Immunogenicity and Thrombocytopenia

- Clinical studies showed development of anti-drug antibodies for ASOs (Mipomersen, Nusinersen, Inotersen)*
- Many ASOs cause thrombocytopenia, which is likely immune-mediated*,#
- Both factors should be considered in the evaluation of new or higher level of product-related impurities in generic drug development

* Labeling information; # Flierl U, et. al., JEM, 2015, 212(2), 129-137.; Sewing S, et. al., PLoS One 2017, 12(11), e0187574

Other Challenges

- Classification of product-related impurities
  - Each type of ON-related impurity contain many structurally different, but similar impurities
- Identification and qualification threshold for new product-related impurities
  - ICH* guidelines on quality do NOT apply to these impurities
- Evaluation of immunogenicity
  - Development of appropriate in vitro assays

*ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Summary

- Both synthetic peptides and ASOs are complex API drugs
- Regulatory thinking on generic synthetic peptide drug development is more mature including recommendations on API sameness demonstration, impurity characterization and immunogenicity risk assessment
- Regulatory thinking on generic synthetic ASOs is still evolving, more research is needed in this area

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FDA Oligonucleotide Research Working Group


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