Session 3

Common Deficiencies and Resolutions: Complex Drug Substances, Complex/Critical Excipients, and Complex Products

Session Leads: Yan Wang, PhD & Dominick Roselle, PhD

Bin Qin, PhD
Senior Staff Fellow
DTPI | ORS | OGD | CDER | FDA

Maotang Zhou, PhD
Division Director
DPQAXVII | OPQAIII | OPQ | CDER | FDA

Sami Nazzal, PhD
Senior Pharmacologist
DTPII | ORS | OGD | CDER | FDA

Keduo Qian, PhD
Chemist
DPQAXIX | OPQAIII | OPQ | CDER | FDA

Jinhui Zhang, PhD
Senior Pharmaceutical Scientist
OPQR | OPQ | CDER | FDA

Dahui Liu, PhD
Senior Pharmaceutical Quality Assessor
DPQAIV | OPQAI | OPQ
| CDER | FDA

Compositional Sameness for Complex Polymeric Excipients: Progress and Remaining Challenges

Bin Qin, PhD
Senior Staff Fellow
DTP I, Office of Research and Standards/OGD
CDER | US FDA

Advancing Generic Drug Development Workshop: Translating Science to Approval
Day 2, Session 3, October 8, 2025

Outlines

- Introduction to compositional sameness requirements
- Case studies: information requirements for complex polymeric excipients
- Conclusions

When is demonstration of compositional sameness needed?

- Regulation[1] requires that generic drugs with parenteral, ophthalmic, and otic routes of administration contain “the same inactive ingredients in the same concentration as the reference listed drug”.
- “same inactive ingredients” is referred as qualitative (Q1) sameness
- “in the same concentration” is referred as quantitative (Q2) sameness

[1] 21 CFR 314.94(a)(9)(iii) and (iv)

Other triggers for demonstration of compositional sameness

In some cases, compositional sameness is recommended to follow bioequivalence studies outlined in the Agency's product-specific guidances (PSGs).

Active Ingredient:	Levonorgestrel
Dosage Form:	System
Route:	Intrauterine
Strength:	19.5 mg
Recommended Studies:	One in vitro bioequivalence study with supportive comparative studies and one in vivo/ex vivo bioequivalence study

To be eligible for the bioequivalence studies recommended in this guidance, the test (T) product should contain no difference in inactive ingredients or in other aspects of the formulation relative to the reference listed drug (RLD) product that may significantly affect the local or systemic availability of the active ingredient. For example, the T product can be qualitatively (Q1) [1] and quantitatively (Q2) [2] the same as the reference standard (RS) to satisfy no difference in inactive ingredients. [3]

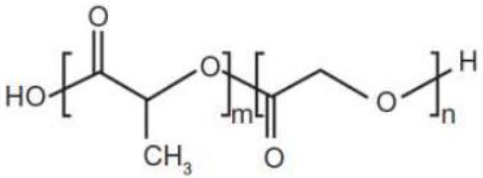
PSG on Levonorgestrel Intrauterine System (NDA 208224)

Q1Q2 evaluation of polymeric components

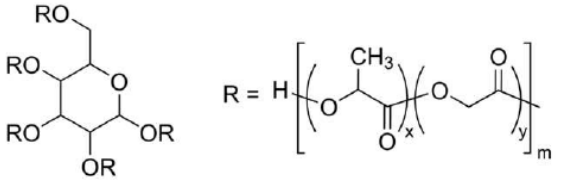
- Straightforward cases:
  - Polyethylene glycol (PEG)
  - Carboxymethylcellulose sodium
  - Ethylene vinyl acetate (EVA) copolymers
- Complex cases requiring additional information
  - Poly(lactide-co-glycolide) (PLGA)
  - Crosslinked hydrogel
  - Polydimethylsiloxane (PDMS) elastomer

Case#1: PLGA

- PLGA is a biodegradable copolymer composed of lactide and glycolide monomers that undergoes hydrolytic degradation in biological environments.
- PLGA functions as the primary rate-controlling excipient in more than 20 FDA-approved long-acting injectable and implantable drug products.
- Key polymer properties include molecular weight and weight distribution, molar ratio of lactide to glycolide, end group, inherent viscosity, glass transition temperature, polymer architecture (linear vs. branched), etc.
- Polymer characteristics can be altered during the manufacturing processes such as microencapsulation, extrusion and sterilization.



Linear PLGA

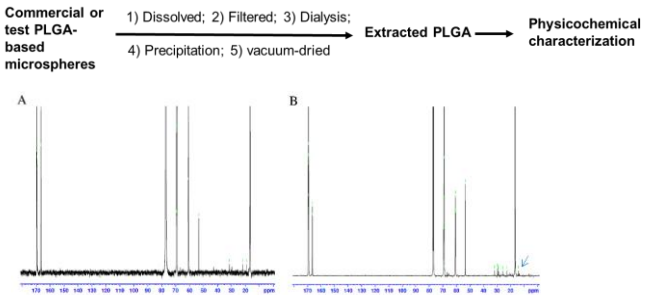


Glucose cored star shaped PLGA

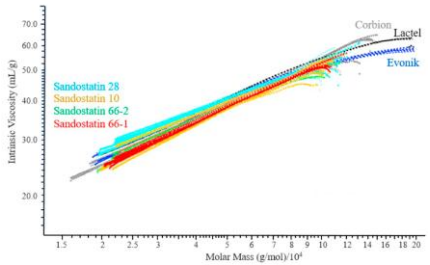
GDUFA research on PLGA characterization

GDUFA[1] research developed:

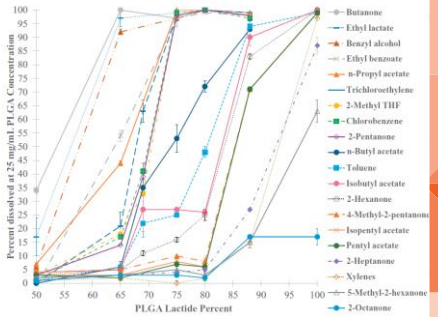
- Extraction and characterization protocol for PLGA analysis from finished drug products
- Advanced characterization methods for glucose-cored, star-shaped PLGA polymers
- Separation techniques for differentiating PLGAs based on different lactide-to-glycolide ratios



Int. J. Pharm. 495 (2015) 87–92
Grant U01FD05168



J. Control. Release 204 (2019) 75-89
Contract HHSF223201710123C



J. Control. Release 300 (2019) 174-184
Contract HHSF223201610091C

[1]Generic Drug User Fee Amendments (GDUFA)

Establish Q1 sameness for PLGA

- To support Q1 sameness of PLGA, provide comparative characterization data including polymer composition (molar ratio between glycolide and lactide), molecular weight and weight distribution, and PLGA architecture (e.g., linear or branched) on the PLGA polymer extracted from the test and the RLD products.
- Branch analysis should be provided for branched PLGA (e.g., glucose cored star shaped PLGA).
- For quality assessment, provide characterization on the extracted PLGA including but not limited to polymer end cap analysis, inherent viscosity, and glass transition temperature.
- For products using PLGA mixture, comparative characterization can be performed using PLGA mixture extracted from the finished products.

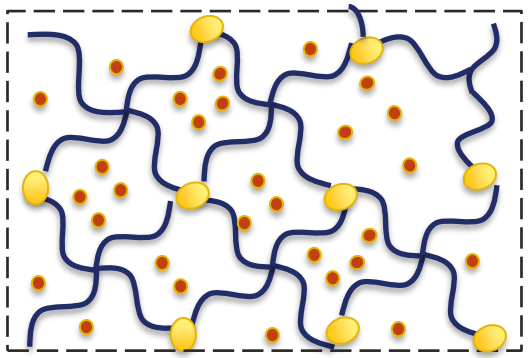
Remaining challenge of PLGA characterization

- Miniaturized drug products present significant practical challenges for reverse engineering and comparative characterization due to their limited material availability.
  - E.g., products such as DURYSTA and OZURDEX are extremely small (<1 mg) [1]
- To address these analytical limitations, GDUFA research is currently being conducted under Contract #75F40123C00192.
- More detailed updates on this research initiative will be presented at the upcoming FDA-CRCG Workshop: Visionary Standards: Advancing Science and Regulation in Generic Ophthalmic Products, November 19-20, 2025.

[1] *Mol Pharm.* 2025, 22(1):446-458

Case#2: Crosslinked hydrogel

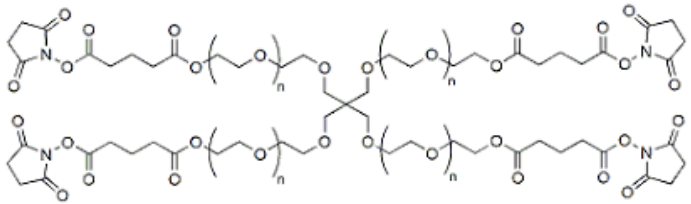
- Hydrogels are three-dimensional polymer networks characterized by high water content and the ability to absorb substantial amounts of aqueous solutions while maintaining their structural integrity.
- Chemical crosslinking methods create covalent bonds between polymer chains, resulting in the formation of stable hydrogel networks.



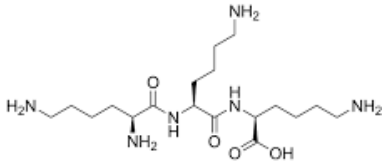
Bioeng Transl Med. 2020;5:e10158.

Example: DEXTENZA (dexamethasone ophthalmic insert)

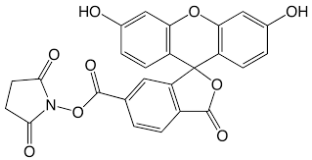
- DEXTENZA is a PEG-based hydrogel made of 4-arm polyethylene glycol (PEG) Nhydroxysuccinimidyl glutarate (20K), trilysine acetate, N-hydroxysuccinimide-fluorescein, sodium phosphate dibasic, sodium phosphate monobasic, water for injection[1].



4 arm PEG NHS



Trilysine



Fluorescein-NHS

- N-hydroxysuccinimde (NHS) ester reacts with primary amine, releasing N-hydroxysuccinimide as a leaving group.

[1]DEXTENZA drug labeling

Establish Q1Q2 sameness for crosslinked PEG-based hydrogel

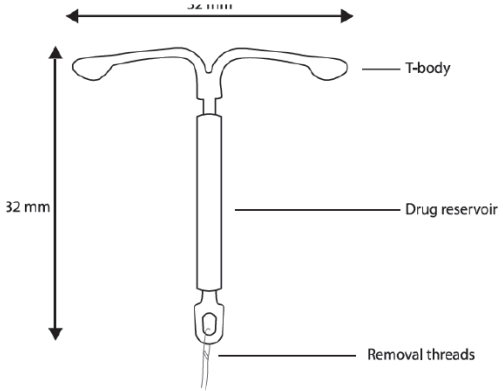
- It is noted that the crosslinked PEG polymers conjugated with fluorescein constitute the actual component in the finished product.
- To support Q1 assessment, applicants should provide detailed description on starting materials, ratios and concentrations of all starting materials and crosslinking method for producing the hydrogel.
- To support Q2 assessment, applicants should provide information on the amount of each starting material. Differences in Q2 could be justified by comparable mechanical properties. In addition, the amount of each starting material should not raise any potential safety concerns.
- An FDA internal research project was conducted to support product-specific guidance development on dexamethasone ophthalmic insert.
- Updates on this research and related regulatory considerations will be presented at the FDA-CRCG Workshop: Visionary Standards: Advancing Science and Regulation in Generic Ophthalmic Products (Nov 19-20, 2025)

Case#3 PDMS

- PDMS elastomers are commonly used as drug reservoir and release controlling excipients in intrauterine systems (IUS) and vaginal rings.
- PDMS is a crosslinked polymer formed by the curing of silicone prepolymers or copolymers and consisted of repeating structural backbone of (-Si-O-).
- Crosslinking methods:
  - Addition curing
  - Condensation curing
  - Peroxide curing
- Pre-polymers kit may contain additive(s).

Int J Pharm. 612 (2022) 121383

Example: MIRENA (levonorgestrel-releasing intrauterine system)



- The reservoir is made of a mixture of levonorgestrel (LNG) and silicone (polydimethylsiloxane).
- The reservoir is covered by a semi-opaque silicone membrane, composed of polydimethylsiloxane and colloidal silica.

MIRENA drug labeling

GDUFA research relevant to PDMS compositional sameness

GDUFA research has developed scientific foundation necessary to evaluate PDMS compositional sameness.

- Development of analytical techniques to measure and characterize PDMS crosslinking density and related material properties
- Understanding of how PDMS crosslinking density affects drug release in IUS (Int J. Pharm. 612 (2022) 121383)
- Impact of curing chemistry (addition curing vs. condensation curing) and fillers on manufacturability and performance of LNG IUS (Int J. Pharm. 660 (2024) 124343)
- Impact of excipients (additives, fillers, lubricants) on formulation attributes and in vitro performance of LNG-IUSs (J Control Release. (2024) 370, 124-139)

Grant 1U01FD005443-01

Compositional sameness of PMDS-based IUS

- To support the Q1Q2 assessment, applicants should provide compositional information (e.g., PDMS chemistry, degree of crosslinking, quantity of each component), dimensional information (e.g., similar length, thickness of membrane) and mechanical properties.

Conclusion

- Demonstrating compositional sameness of a polymeric excipient is generally straightforward, but complex cases exist that require additional information such as comparative characterization to support sameness evaluation.
- In these complex cases, GDUFA-funded research has developed the analytical methods and scientific foundation necessary to support sameness evaluation.

Questions?

Bin Qin
Senior Staff Fellow
DTP I, Office of Research and Standards/OGD
CDER | US FDA

Navigating Complexity:
Key Considerations in Developing the Oral Semaglutide Product-Specific Guidance

Sami Nazzal, Ph.D.
Senior Pharmacologist
Division of Therapeutic Performance II
Office of Research and Standards
Office of Generic Drugs
CDER | US FDA

Advancing Generic Drug Development Workshop: Translating Science to Approval

Session 3: Common Deficiencies and Resolutions: Complex Drug Substances, Complex/Critical Excipients, and Complex Products
October 8, 2025

Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA's official views or policies.

Outline

I. Background: Oral semaglutide

II. Product-specific guidance (PSG) evolution from 2021: Key changes

III. Analytical challenges and future directions

I. Background: Oral semaglutide

First and the only oral glucagon-like peptide-1 (GLP-1) receptor agonist

Indication	Improve glycemic control in adults with type 2 diabetes
R1 Approval	3 mg, 7 mg, 14 mg strengths approved September 2019
R2 Approval	1.5 mg, 4 mg, 9 mg strengths approved December 2024
Key Formulation Attributes	R1 and R2 require absorption enhancer (salcaprozate sodium [SNAC]) due to low oral bioavailability
PSG Status/ Revisions	Draft PSG published August 2021 for Formulation R1 Revised PSG** published October 2025 for Formulations R1 and R2

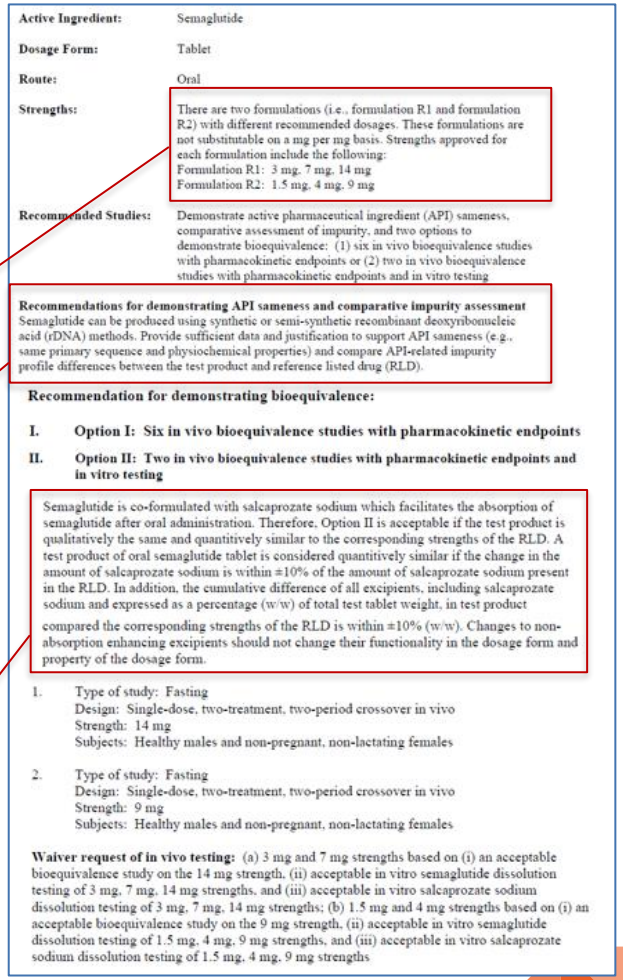
*Code of Federal Regulations: 21 CFR 600.3
**Draft Guidance on Semaglutide Oral Tablet (10/01/2025)

II. PSG Evolution from 2021 to 2025: Key changes

Change # 1
Expanded Study Recommendations for New Strengths

Change # 2
Added Active Pharmaceutical Ingredient (API) Sameness & Impurity Assessment

Change # 3
Clarified Quantitative Criteria for Option II



Active Ingredient: Semaglutide

Dosage Form: Tablet

Route: Oral

Strengths: There are two formulations (i.e., formulation R1 and formulation R2) with different recommended dosages. These formulations are not substitutable on a mg per mg basis. Strengths approved for each formulation include the following:
Formulation R1: 3 mg, 7 mg, 14 mg
Formulation R2: 1.5 mg, 4 mg, 9 mg

Recommended Studies: Demonstrate active pharmaceutical ingredient (API) sameness, comparative assessment of impurity, and two options to demonstrate bioequivalence: (1) six in vivo bioequivalence studies with pharmacokinetic endpoints or (2) two in vivo bioequivalence studies with pharmacokinetic endpoints and in vitro testing

Recommendations for demonstrating API sameness and comparative impurity assessment
Semaglutide can be produced using synthetic or semi-synthetic recombinant deoxyribonucleic acid (rDNA) methods. Provide sufficient data and justification to support API sameness (e.g., same primary sequence and physiochemical properties) and compare API-related impurity profile differences between the test product and reference listed drug (RLD).

Recommendation for demonstrating bioequivalence:

I. **Option I: Six in vivo bioequivalence studies with pharmacokinetic endpoints**

II. **Option II: Two in vivo bioequivalence studies with pharmacokinetic endpoints and in vitro testing**

Semaglutide is co-formulated with salcaprozate sodium which facilitates the absorption of semaglutide after oral administration. Therefore, Option II is acceptable if the test product is qualitatively the same and quantitively similar to the corresponding strengths of the RLD. A test product of oral semaglutide tablet is considered quantitively similar if the change in the amount of salcaprozate sodium is within ±10% of the amount of salcaprozate sodium present in the RLD. In addition, the cumulative difference of all excipients, including salcaprozate sodium and expressed as a percentage (w/w) of total test tablet weight, in test product compared the corresponding strengths of the RLD is within ±10% (w/w). Changes to non-absorption enhancing excipients should not change their functionality in the dosage form and property of the dosage form.

1. Type of study: Fasting
   Design: Single-dose, two-treatment, two-period crossover in vivo
   Strength: 14 mg
   Subjects: Healthy males and non-pregnant, non-lactating females

2. Type of study: Fasting
   Design: Single-dose, two-treatment, two-period crossover in vivo
   Strength: 9 mg
   Subjects: Healthy males and non-pregnant, non-lactating females

Waiver request of in vivo testing: (a) 3 mg and 7 mg strengths based on (i) an acceptable bioequivalence study on the 14 mg strength, (ii) acceptable in vitro semaglutide dissolution testing of 3 mg, 7 mg, 14 mg strengths, and (iii) acceptable in vitro salcaprozate sodium dissolution testing of 3 mg, 7 mg, 14 mg strengths; (b) 1.5 mg and 4 mg strengths based on (i) an acceptable bioequivalence study on the 9 mg strength, (ii) acceptable in vitro semaglutide dissolution testing of 1.5 mg, 4 mg, 9 mg strengths, and (iii) acceptable in vitro salcaprozate sodium dissolution testing of 1.5 mg, 4 mg, 9 mg strengths

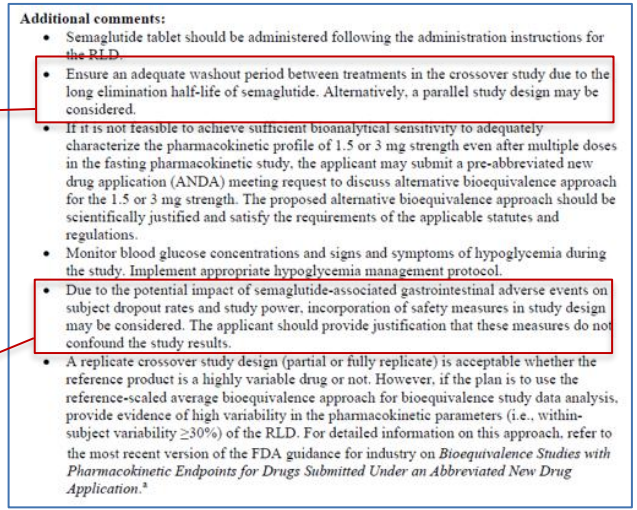
Draft Guidance on Semaglutide Oral Tablet (10/01/2025)

Change # 4

Washout Period Recommendations Addressing Long Half-Life Challenges

Change # 5

Gastrointestinal (GI) Adverse Events and Safety Measures



Additional comments:

- Semaglutide tablet should be administered following the administration instructions for the RLD.
- Ensure an adequate washout period between treatments in the crossover study due to the long elimination half-life of semaglutide. Alternatively, a parallel study design may be considered.
- If it is not feasible to achieve sufficient bioanalytical sensitivity to adequately characterize the pharmacokinetic profile of 1.5 or 3 mg strength even after multiple doses in the fasting pharmacokinetic study, the applicant may submit a pre-abbreviated new drug application (ANDA) meeting request to discuss alternative bioequivalence approach for the 1.5 or 3 mg strength. The proposed alternative bioequivalence approach should be scientifically justified and satisfy the requirements of the applicable statutes and regulations.
- Monitor blood glucose concentrations and signs and symptoms of hypoglycemia during the study. Implement appropriate hypoglycemia management protocol.
- Due to the potential impact of semaglutide-associated gastrointestinal adverse events on subject dropout rates and study power, incorporation of safety measures in study design may be considered. The applicant should provide justification that these measures do not confound the study results.
- A replicate crossover study design (partial or fully replicate) is acceptable whether the reference product is a highly variable drug or not. However, if the plan is to use the reference-scaled average bioequivalence approach for bioequivalence study data analysis, provide evidence of high variability in the pharmacokinetic parameters (i.e., within-subject variability ≥30%) of the RLD. For detailed information on this approach, refer to the most recent version of the FDA guidance for industry on *Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an Abbreviated New Drug Application*.[8]

Change # 1
Expanded Study Recommendations for New Strengths

2025 Change: Option I

Six bioequivalence studies recommended for Option I (both R1 and R2 formulations) vs. three studies in 2021

- 2021: 3 fasting BE studies for Option I (Formulation R1 only)
- 2025: 6 fasting BE studies (Both formulations)
  - Formulation R1: 14 mg, 7 mg (single dose), 3 mg (multiple dose)
  - Formulation R2: 9 mg, 4 mg (single dose), 1.5 mg (multiple dose)

Rationale

- R1 Approved in Sep 2019, R2 Approved in Dec 2024
- "These formulations are not substitutable on a mg per mg basis”*
- Oral bioavailability differences: R1 (0.4-1%) vs R2 (1-2%)

Drugs@FDA. RYBELSUS® (semaglutide) tablets, for oral use (12/2024)

Change # 2
Added API Sameness & Impurity Assessment

2025 Change

Added new language: "Semaglutide can be produced using synthetic or semi-synthetic recombinant deoxyribonucleic acid (rDNA) methods."

Rationale

1	Multiple controlled correspondences questioned whether rDNA-derived semaglutide qualified for 505(j) ANDA pathway given existing peptide guidance*
2	May 2021 peptide guidance addressed injectable peptides
3	Oral route of administration is generally associated with lower immunogenicity. Furthermore, low oral bioavailability of semaglutide attenuates the risk of immune responses relative to injectable formulations, thereby permitting greater flexibility in the manufacturing approaches for oral products.
4	2025 PSG clarifies that both synthetic and rDNA manufacturing methods are acceptable for ANDA pathway

FDA Guidance: "ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin," May 2021

Change # 3

Clarified Quantitative Criteria for Option II

(fasting BE studies on 9 mg and 14 mg. Waiver request for lower strengths)

- 2021 PSG Option II recommended products be qualitatively the same and **quantitively similar** to the RLD without defining "similar," creating uncertainty in formulation assessment

Background:

- Qualitatively the same = same inactive ingredients
- Quantitively the same = within +/- 5% of RLD concentration*
- Quantitively similar: no formal definition exists

*ANDA Submissions - Refuse-to-Receive Standards Guidance, December 2016

2025 Change

Added specific criteria for similarity assessment for oral semaglutide tablet: **A test product is considered quantitively similar if**:

- The amount of SNAC (which facilitates oral absorption) is within ±10% of the amount of SNAC in the RLD
- Cumulative difference of all excipients (including SNAC), expressed as % (w/w) of total test tablet weight, is within ±10% (w/w) compared to the RLD

Rationale

- 2025 PSG provides clear evaluation criteria, creating enhanced guidance that supports standardized assessment approaches

Change # 4

Washout Period Recommendation Addressing Long Half-Life Challenges

2025 Change

Added new language: "Ensure an adequate washout period between treatments in the crossover study due to the long elimination half-life of semaglutide. Alternatively, a parallel study design may be considered."

Rationale

- Semaglutide exhibits ~1 week elimination half-life with drug present for about 5 weeks after last dose
- FDA's BE guidance* recommends a washout that is 5x the drug’s half-life

*FDA Guidance: "Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA," August 2021

Change # 5

GI Adverse Events and Safety Measures

2025 Change

Added new language: “Due to the potential impact of semaglutide-associated gastrointestinal adverse events on subject dropout rates and study power, incorporation of safety measures in study design may be considered."

Rationale*

- GI adverse events (nausea, vomiting) are well documented with semaglutide use
- High dropout rates in bioequivalence studies can compromise study validity and statistical power

*Shu Y, He X, Wu P, Liu Y, Ding Y, Zhang Q. Gastrointestinal adverse events associated with semaglutide: A pharmacovigilance study based on FDA adverse event reporting system. Front Public Health. 2022;10:996179.

Study Design Considerations for GI Adverse Events

1	Titration schemes may reduce adverse events but increase study duration and participant exposure.
2	Safety measures, including antiemetics, may be considered to manage vomiting and reduce dropout rates.
3	Antiemetics may affect gastric motility and drug absorption and may complicate bioanalytical methods.
4	Each approach must be justified to demonstrate it does not confound study results.

III. Analytical challenges and future directions

Alternative Absorption Enhancement Strategies

- Develop bioequivalence recommendations for non-SNAC formulations
- Create waiver pathways for products using different enhancement strategies

Advanced Analytical Methods

- Improved lower limit of quantification (LLOQ) methods may enable single-dose studies for 1.5 mg and 3 mg strengths
- Improved analytical techniques to better handle SNAC matrix effects
- Enable more precise characterization of peptide-related impurities for better API sameness assessment

Thank you!

Key Considerations in Developing Oral Semaglutide Products

Jinhui Zhang, Ph.D.

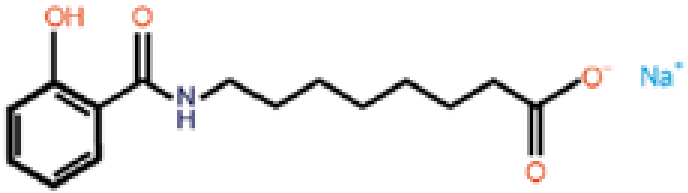
Senior Pharmaceutical Scientist
Office of Pharmaceutical Quality Research, Office of Pharmaceutical Quality
CDER | US FDA

Advancing Generic Drug Development Workshop: Translating Science to Approval
October 7-8, 2025

Outline

- Insights form pharmaceutical development of oral semaglutide
- Strategies for impurities profiling and quantitation
- Bioanalytical challenges to demonstrate bioequivalence
- More questions to be addressed……

Insights form pharmaceutical development



The Key Excipient: SNAC

- Absorption in stomach
  - *Enhancing permeation*
  - *Preventing oligomerization*
  - *Buffering effect*
- Delay of gastric emptying

	Strength (mg)	Other excipients
Formulation “R1” (2019)	3/7/14	Magnesium stearate CMC, Povidone K90
Formulation :R2” (2024)	1.5/4/9	Magnesium stearate

Equivalent!!!

https://pubmed.ncbi.nlm.nih.gov/30429357/
https://pubmed.ncbi.nlm.nih.gov/39708086/
https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/213051s018lbl.pdf

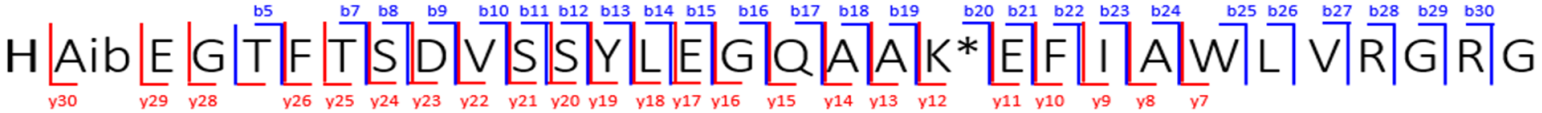
Challenges for oral semaglutide products

- Specific issues with tablet dosage form
  - Extraction efficiency, interferences and matrix effect for assay and impurities
  - LC-MS bioanalytical methods in complex matrixes for *in vivo* BE
  - Discriminating capability of dissolution methods for *in vitro* BE
- Q1Q2 sameness for ANDA(s): challenges for tablet still apply, and
  - What are critical processing parameters?
  - API-excipient interaction mechanisms inform *in vivo* drug performance evaluation design
  - API-excipient interaction mechanisms inform *in vitro* drug performance evaluation design
- If Q1Q2 *non sameness*: challenges for tablet still apply, and
  - API-excipient interaction mechanisms might necessitate different regulatory pathway

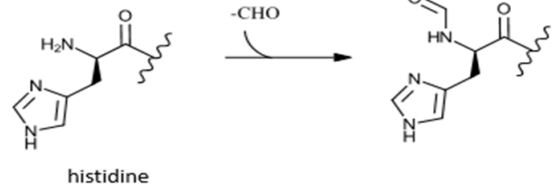
Strategies for impurities profiling and quantitation per ICH Q14 and Q2(R2) recommendations

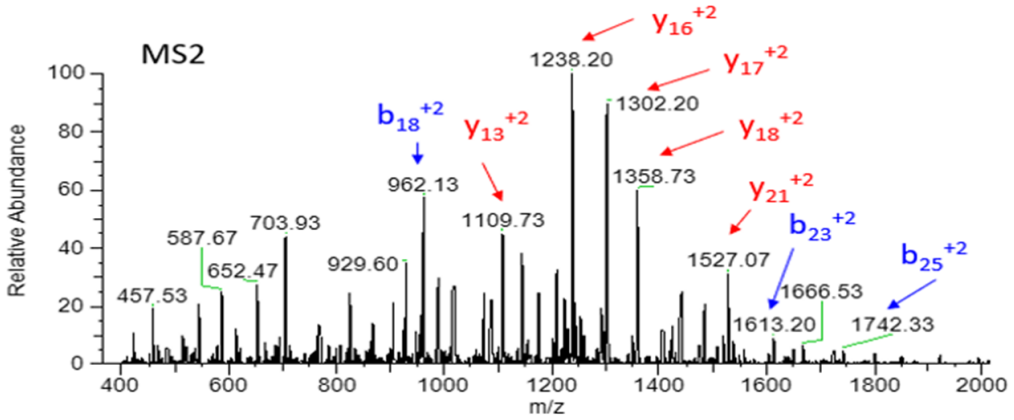
- Limited prior knowledge on detailed structural elucidation
  - MS/MS characterization at intact form eliminating the needs for fraction collection and digestion.
  - Combinational using of multiple fragmentation mechanisms to achieve high sequence coverage.
- No commercially available standards for method development
  - In-house prepared heat-stressed injectable semaglutide as positive control and “surrogate standards”.
- Extraction efficiency for oral semaglutide products
  - Using sample solution in the submission as starting point.
  - In-depth understanding of physical and chemical characteristics of all excipients.
- Potential interferences form excipients
  - Screening different lots of excipients prior to method validation
- Potential matrix effect from tablets extraction impacting accuracy
  - Spiking semaglutide standard to placebo tablets extraction as routine evaluation of matrix effect.
  - Spiking *internal standard* to semaglutide tablets extract to gain real world evidence of matrix effect.
- Analytical variables associated with study samples
  - Using internal standard to track matrix effect per ICH Q2(R2) guideline recommendation.
  - Monitoring multiple ions to ensure specificity per ICH Q2(R2) recommendation.
- Quantitation of unknown impurities
  - Using extracted ion chromatograms of multiple ions as readout.
  - Parallelism approaches to investigate matrix effect if necessary.
- Sensitivity
  - Using alternative MS data acquisition algorithms to achieve greater sensitivity.

Example of impurity structural elucidation: formyl-histine 1

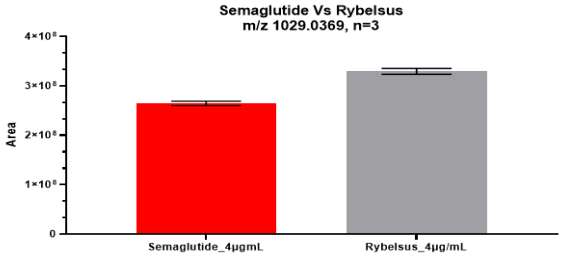


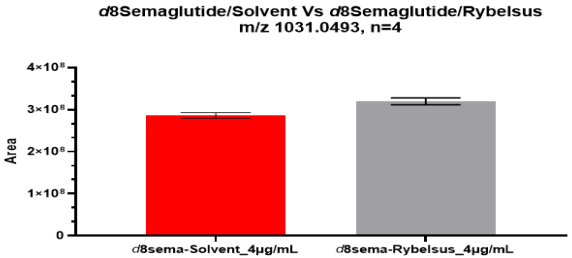
In-House data b-Ion Coverage	b-Ion	Semaglutide Sequence	y-Ion	In-House data y-Ion Coveraged
-	b1	H-formylation	y31	-
-	b2	Aib	y30	Aib
-	b3	E	y29	E
-	b4	G	y28	G
T	b5	T	y27	-
-	b6	F	y26	F
T	b7	T	y25	T
S	b8	S	y24	S
D	b9	D	y23	D
V	b10	V	y22	V
S	b11	S	y21	S
S	b12	S	y20	S
Y	b13	Y	y19	Y
L	b14	L	y18	L
E	b15	E	y17	E
G	b16	G	y16	G
Q	b17	Q	y15	Q
A	b18	A	y14	A
A	b19	A	y13	A
K*	b20	K*	y12	K*
E	b21	E	y11	E
F	b22	F	y10	F
I	b23	I	y9	I
A	b24	A	y8	A
W	b25	W	y7	W
L	b26	L	y6	-
V	b27	V	y5	-
R	b28	R	y4	-
G	b29	G	y3	-
-	b30	R	y2	-
G	b31	G	y1	-

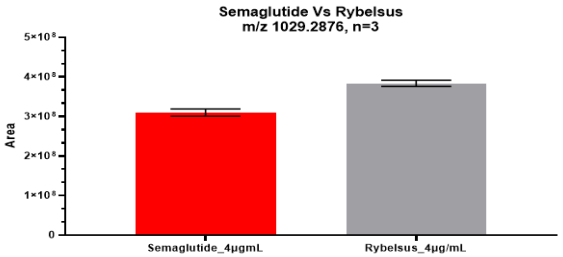


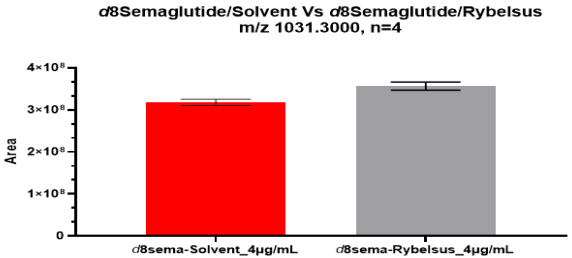


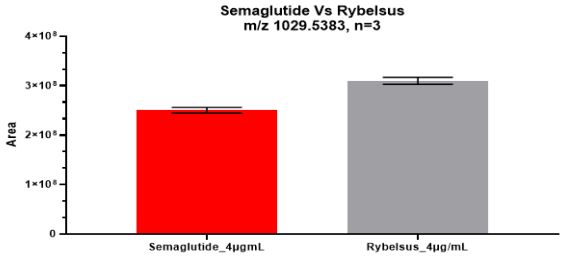
Evaluating extraction efficiency and matrix effect per ICH Q2(R2) recommendation

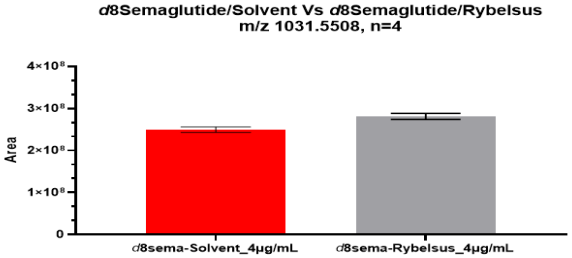








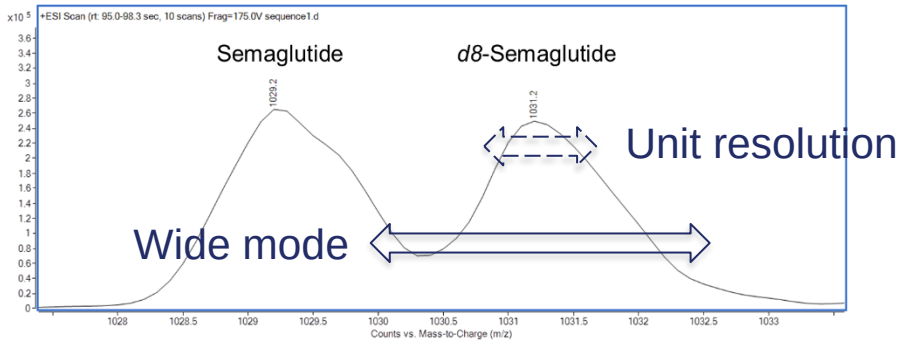




Bioanalytical challenges to demonstrate bioequivalence

The devil is in the details!!!

- Sensitivity
- Selection of ions
- Internal standard (s)
- Non-specific binding
- Stability



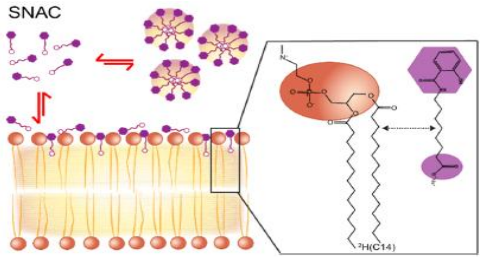
The **early pharmaceutical development** of oral semaglutide product might have been **hampered by matrix interference** in the incurred samples for a validated ligand binding method used for quantitating semaglutide in biological matrixes!

https://pubmed.ncbi.nlm.nih.gov/30429357/

https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/213051Orig1s000ClinPharmR.pdf

More questions to be addressed

- Is there direct and specific interaction between SNAC and semaglutide?

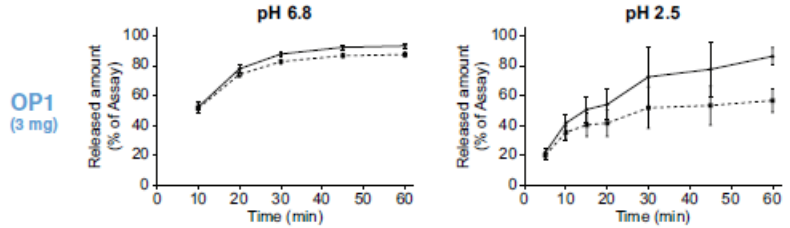


- Are there differences between synthetic semaglutide?
- Impact of buffer and pH on semaglutide stability?

https://pubmed.ncbi.nlm.nih.gov/39690106/
https://pubmed.ncbi.nlm.nih.gov/40490042/
https://pubmed.ncbi.nlm.nih.gov/36592951/

More questions to be addressed…

- How to ensure the discriminating ability of dissolution method?



- Should the clinical study design take into consideration gastric emptying, delaying the effect of semaglutide?

https://pubmed.ncbi.nlm.nih.gov/39379664/
https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/213051s018lbl.pdf

Acknowledgment

Office and Division Management
Patrick J. Faustino
Muhammad Ashraf
Xiaoming Xu
Thomas O'Connor
David Keire

Scientific Communities
Arne Staby
Marloes van Hout
Yongchao Su
Jing Ling
Chengjie Ji
Shanshan Guan

FDA
Paresma (Pinky) Patel
Chongwoo Yu
Eric S. Pang
Heather J. Boyce
Wei-Jhe Sun
Sami Nazzal
Yan Wang
Katherine Windsor
Kang Chen
Roberto Mendez
Nobel O. Sierra-Vega
Mack Shih
Jianghong (Grace) Gu
Guozhang Zou
Robert L. Hunt
Robert M. Lupitskyy

U.S. FOOD & DRUG ADMINISTRATION

Questions?

Case Studies:
Glatiramer Acetate (GA) Injection

Maotang Zhou, PhD
Division Director, OPQAXVII
OPQAIII, Office of Pharmaceutical Quality (OPQ)
CDER | US FDA

Advancing Generic Drug Development Workshop: Translating Science to Approval
October 7-8, 2025

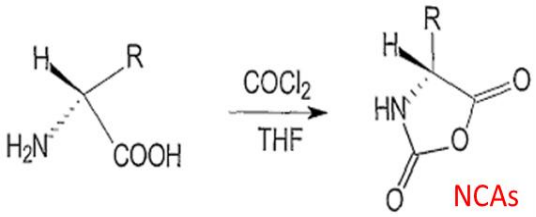
Outline

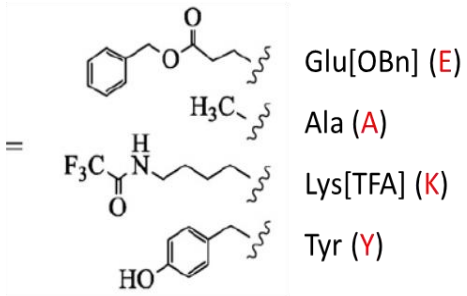
- Introduction
- Establishment of API Sameness
- Common deficiencies
- Conclusion

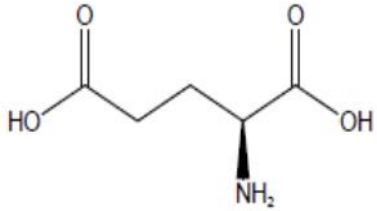
Introduction: GA Injection

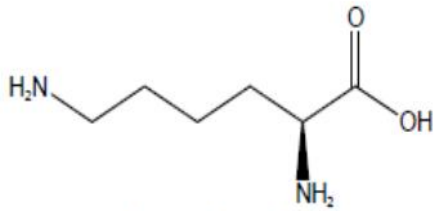
- **RLD**: Copaxone® (glatiramer acetate injection)
- **Indication**: Multiple Sclerosis
- **API**: a mixture of copolymers of 4 amino acids
  - **AA**: Glu (E), Lys (L), Ala (A), and Tyr (Y)
  - **Average molar faction**: 0.141, 0.338, 0.427, 0.095
  - **Average MW**: 5 – 9 kDa

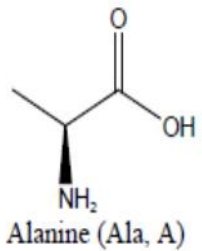
NCA-Amino Acids for GA

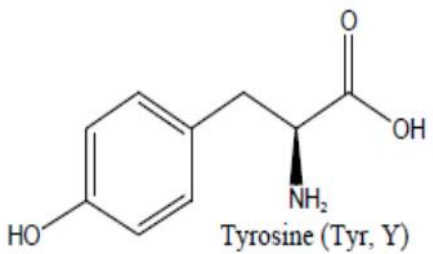






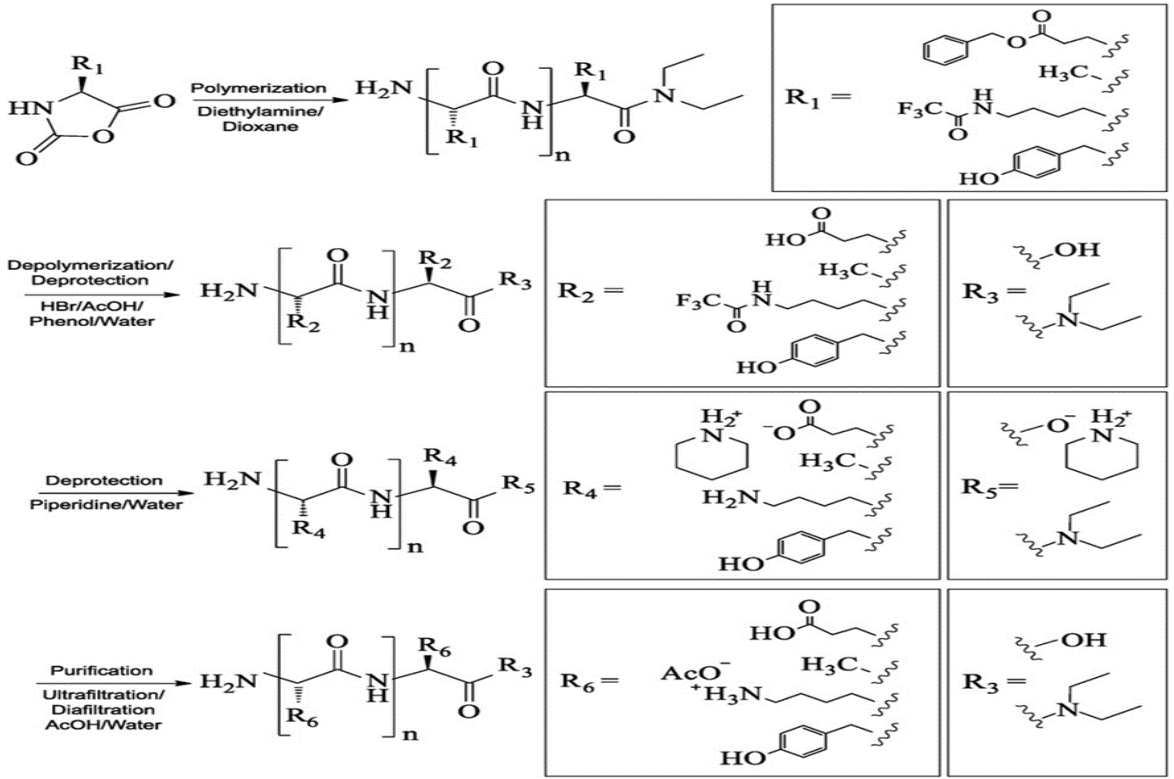






N-Carboxyanhydride (NCA) Amino Acids

GA Synthetic Scheme



J. Anderson, et al., J. Neurological Sci., 359 (2015) 24-34.

GA Copolymer: Key Features

- Conserved Characteristics of copolymer chain
  - Neither entirely conserved, nor completely random
- Batch-to-batch Variability
  - Sequence variations across copolymer chain, coupled with conservation of “local sequences”
- GA active pharmaceutical ingredient (API) is a complex mixture of copolymers, not a single small molecule
  - Requiring the **“Totality of Evidence”** approach to demonstrate the API sameness

Why API Sameness?

- An FDA-approved generic drug is therapeutically equivalent to the reference listed drug (RLD)
  - Bioequivalence
  - Pharmaceutical equivalence
- What is Pharmaceutical Equivalence?
  - Same active pharmaceutical ingredients (APIs)
  - Same dosage form, route of administration, and labeling
  - To produce the same clinical effect and safety profile
- ANDA can't be approved without **API sameness**

Demonstrating GA API Sameness

- Equivalence of Fundamental Reaction Scheme
  - Same NCA-amino acids, initiator, reagents for cleavage
- Equivalence of Physicochemical Properties
  - AA content, MW distribution, spectroscopic fingerprints
- Equivalence of Structural Signatures
  - Process signatures of polymerization/depolymerization
- Equivalence of Biological Assay Results
  - Confirmatory evidence of API sameness

Common Deficiencies

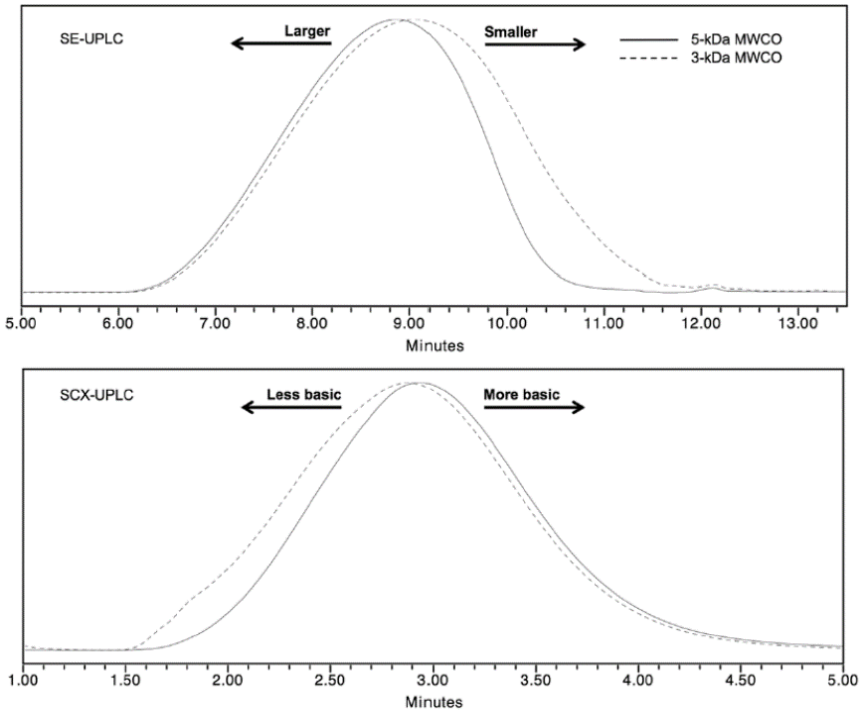
- Inadequate RLD characterization link to “API sameness”
  - Batch selection, # of batches, tests, and equivalence criteria
- Insufficient process understanding link to “sameness”
  - Polymerization (initiation, propagation), depolymerization, purification (**diafiltration**).
- Inadequate data on structural signatures
  - Proper identification and characterization
- Improper negative control studies
  - To challenge sensitivity & specificity of characterization methods

US FDA. Response to Citizen Petition, Docket No. FDA-2015-P-1050. http://www.regulations.gov (2015).

Ultrafiltration/Diafiltration (UF/DF)

- A purification step for crude GA mixture after depolymerization process
  - To remove process impurities (i.e., reagents, salts, etc.)
- Some low MW peptide components may be removed
  - Dependent on process conditions (e.g., MWCO)
- It is crucial to understand how the UF/DF process could impact structural signatures for API sameness

Impact of UF/DF Process



- **MWCO** – Molecular weight cutoff
- **SE-UPLC**: Size exclusion UPLC
- **SCX-UPLC**: Strong cation exchange UPLC

Campos-García, V. R., et al., Sci. Rep., 2017, 7(1), 12125.

Impact on Structural Signatures

- UF/DF process should not alter structural signatures of individual peptide molecules
- UF/DF could impact structural signature characterization based on collective analysis
- Appropriate techniques (e.g., fractionation studies) can be used to study the impact

Summary

- GA, a complex mixture of copolymers
- Totality of Evidence for API sameness
- Structural signatures
- Impact of UF/DF process

Resources

1. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/020622s116lbl.pdf. 2023;Copaxone Package Insert.
2. https://www.accessdata.fda.gov/drugsatfda_docs/psg/PSG_020622.pdf. 2023;Draft Guidance on Glatiramer Acetate.
3. https://www.regulations.gov/document/FDA-2015-P-1050-0012. 2015;Citizen Petition Denial Letter From CDER to Teva Pharmaceuticals.
4. Anderson J, Bell C, Bishop J, Capila I, Ganguly T, Glajch J, et al. Demonstration of equivalence of a generic glatiramer acetate (Glatopa™). Journal of the Neurological Sciences. 2015;359(1):24-34.
5. https://www.chromatographyonline.com/view/molecular-weight-analysis-glatiramer-acetate-and-related-compounds-size-exclusion-chromatography-mul. 2020;38(2).
6. Campos-García VR, Herrera-Fernández D, Espinosa-de la Garza CE, González G, Vallejo-Castillo L, Avila S, et al. Process signatures in glatiramer acetate synthesis: structural and functional relationships. Sci Rep. 2017;7(1):12125.
7. Raw AS, Wu L. Scientific Considerations in the Approval of Complex Generics. In: Sasisekharan R, Lee SL, Rosenberg A, Walker LA, editors. The Science and Regulations of Naturally Derived Complex Drugs. Cham: Springer International Publishing; 2019. p. 157-73
8. Zelzer M, Heise A. Determination of copolymerisation characteristics in the N-carboxy anhydride polymerisation of two amino acids. Polymer Chemistry. 2013;4(13):3896-904.
9. Sarah Rogstad, Eric Peng, Cynthia Sommers, Meng Hu, Xiaohui Jiang, David Keire, Michael T Boyne, Modern analytics for synthetically derived complex drug substances: NMR, AFFF–MALS, and MS tests for glatiramer acetate, Anal Bioanal Chem. (2015) DOI 10.1007/s00216-015-9057-8.

Case Studies: Pentosan Polysulfate Sodium (PPS) Oral Capsules

Keduo Qian, PhD
Chemist, OPQAXIX
OPQAIII, Office of Pharmaceutical Quality (OPQ)
CDER | US FDA

Advancing Generic Drug Development Workshop: Translating Science to Approval
October 7-8, 2025

Introduction: PPS Oral Capsules

- **RLD**: Elmiron® (Pentosan Polysulfate Sodium Capsule, 100 mg)
  - Approved on 09/26/1996
  - The recommended dose of Elmiron® is 300 mg/day taken as one 100 mg capsule orally three times daily.
- **Indication**: Relief of bladder pain or discomfort associated with interstitial cystitis.
- **API**: a semi-synthetically produced heparin-like macromolecular carbohydrate derivative, which chemically and structurally resembles glycosaminoglycans.
  - **Average MW**: 4000 to 6000 Dalton

Pentosan Polysulfate Sodium (PPS)

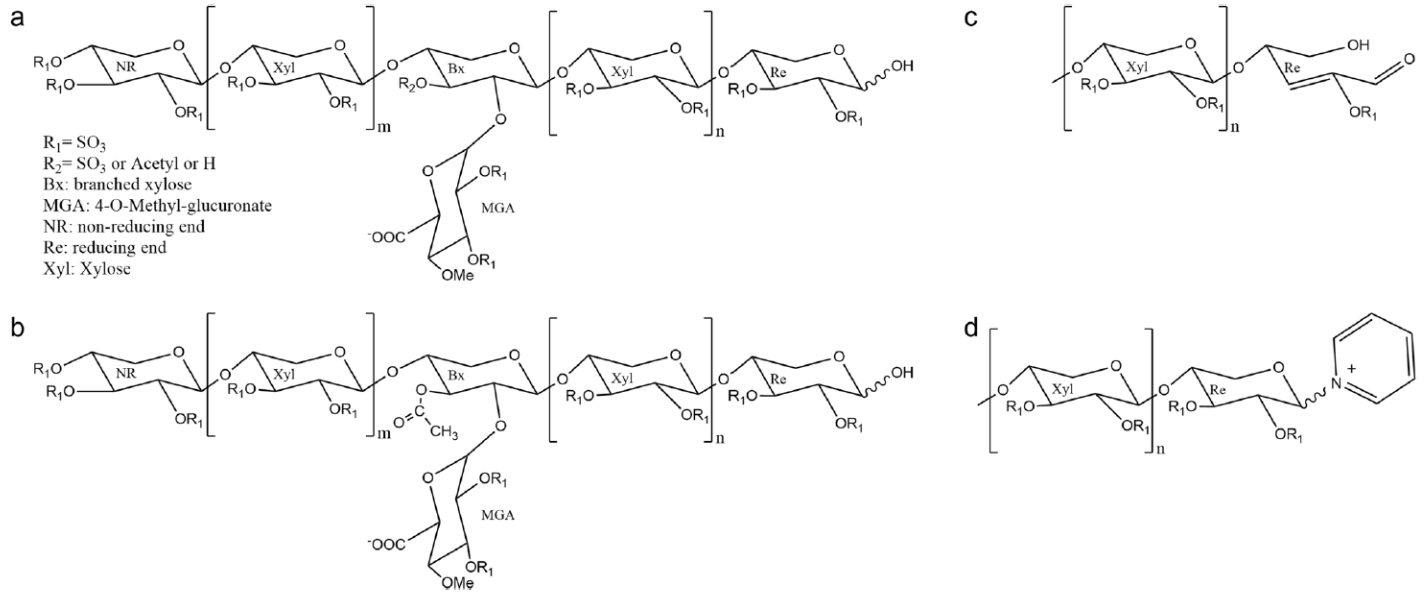


Fig. 1 The chemical structure of pentosan polysulfate sodium (PPS) backbone (**a**) and its modifications of acetylation (**b**) in branched xylose, aldehyde (**c**) and pyridine (**d**) addition at the reducing ends. Integer numbers m and n may take values from 0 to 26 (22)

Wang K et al. The AAPS Journal (2023) 25:50 https://doi.org/10.1208/s12248-023-00815-4

Demonstrating PPS API Sameness

- Equivalence of source of naturally-occurring starting material
- Equivalence of physicochemical properties
  - MW distribution, sulfation degree, sodium content, etc
- Equivalence of the monosaccharide building block composition and chain branching
  - Xylose units, sulfation pattern, glucuronic acid groups, linkages, anomeric configurations, etc
- A comprehensive characterization of impurity profile

Product Specific Guidance (PSG) on Pentosan Polysulfate Sodium: Recommended Sep 2012; Revised Jul 2014, May 2021.

Source of naturally-occurring starting material

- The starting material used to manufacture the proposed PPS should be the same as that used to manufacture the drug substance for the RLD
  - Botanical raw material (BRM) identity
    - The same plant species (e.g. DNA barcoding)
    - The plant parts (heartwood, sapwood, or outer bark etc.) used as the BRM should be defined and be consistent through the life cycle of the product
  - The starting material is the extracted Xylan

Primary Characterization Methods for Sameness Study

- NMR (1H NMR, 13C NMR, 2D COSY, 2D HSQC, etc) – sulfation pattern, reducing end composition, glucuronic acid position and content, linkages, and anomeric configurations, etc
- ICP-MS and IC for compositional analysis of sodium, sulfate, etc
- CHNS elemental analysis
- MW distribution by Gel permeation chromatography (GPC) and polydispersity (PD) comparison
- Monosaccharide building block composition & Polysaccharide chain mapping by chromatography
- Raman and FT-IR spectra
- UV-Vis spectrum
- Polarimetry (specific optical rotation)

Common Deficiencies

- Inadequate RLD information
  - Batch selection, # of batches, tests, and equivalence criteria
- Insufficient process understanding
  - Structural signatures are molecular fingerprints left by the manufacturing process
- Inappropriate classification of minor components vs impurities
  - O-acetylation, PPS-pyridine, PPS-aldehyde
- Inadequate data on structural signatures
  - Proper identification and characterization

PPS Monosaccharide content analysis

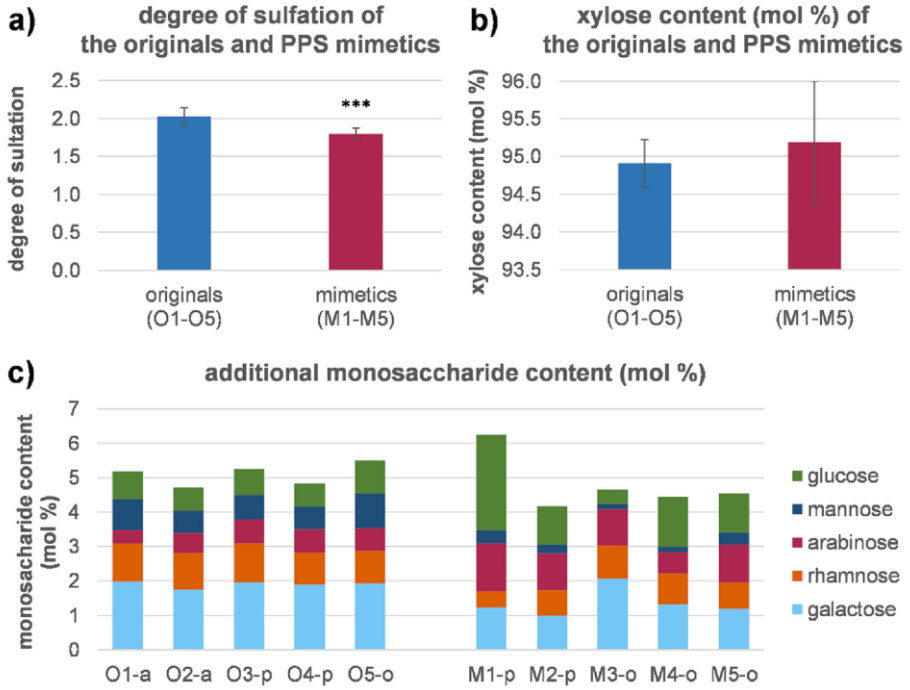


Fig. 6. Degree of sulfation and monosaccharide contents of PPS samples. a) degree of sulfation of the original and mimic PPS samples (***, $P < 0.001$); b) xylose content (mol %) of the original and mimic PPS samples; a) and b) mean ± SD, $n = 2$; c) contents (mol %) of additional monosaccharides of the PPS samples (mean, n = 2).

D. Lenhart, M.-T. et al. *Carbohydrate Polymers* 2023, *319*, 121201, DOI: https://doi.org/10.1016/j.carbpol.2023.121201

PPS Minor Components

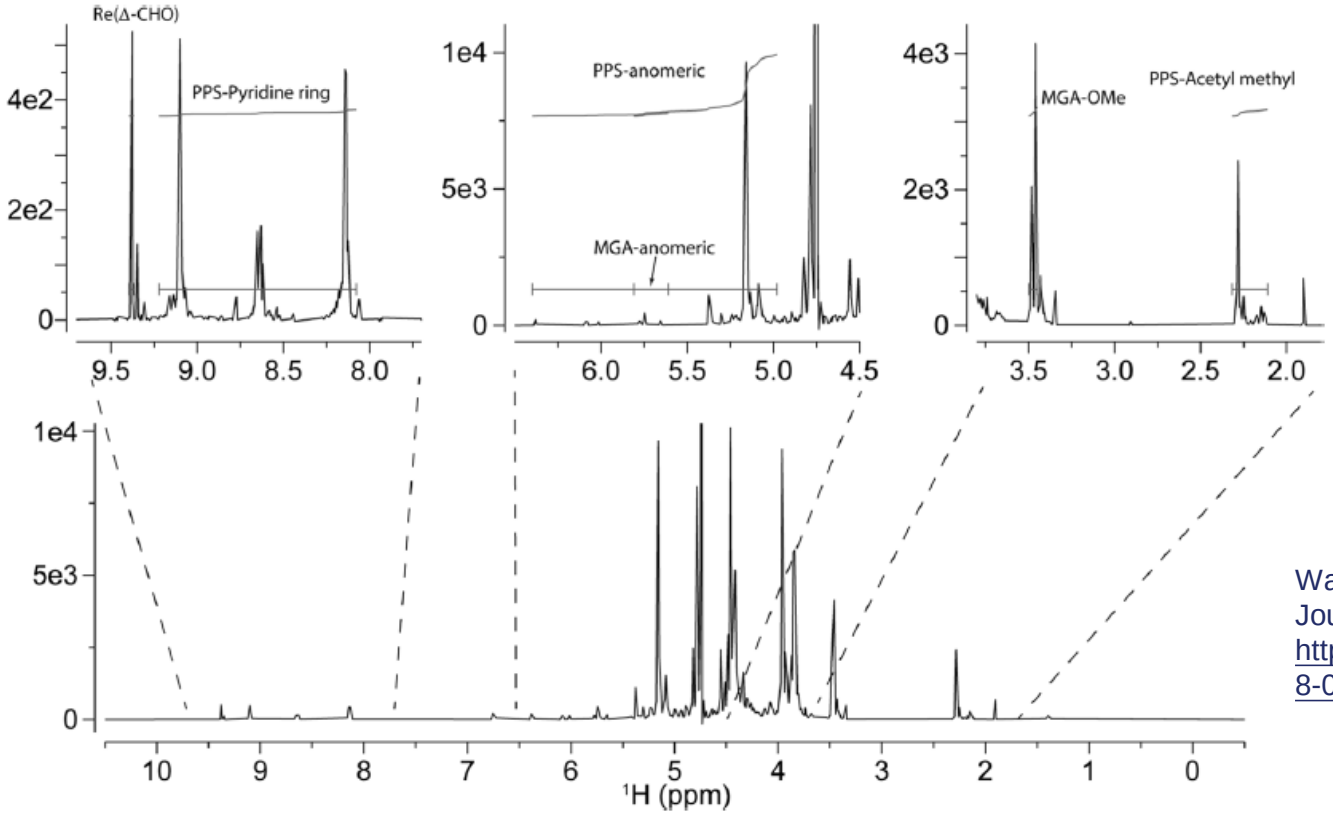


Fig. 2 The representative PPS 1D ^{1}H qNMR spectrum with integration ranges shown in the three sub-spectra

Wang K et al. The AAPS Journal (2023) 25:50 https://doi.org/10.1208/s12248-023-00815-4

PPS Minor Components

	MGA(OMe)%	MGA(anomeric)%	Re(Δ-CHO)%	PPS-Ac%	PPS-Py%	D_{nmr} (cm^2s^{-1})
Lot#1	4.919	5.157	0.372	2.938	1.781	1.18e-6
Lot#2	4.581	4.963	0.506	3.404	2.111	1.23e-6
Lot#3	4.808	5.053	0.489	3.333	2.123	1.20e-6
Lot#4	4.868	4.971	0.452	3.056	2.136	1.18e-6
Lot#5–1	4.776	5.080	0.550	3.487	2.021	1.29e-6
Lot#6	4.878	5.014	0.540	3.466	2.017	1.33e-6
Mean[a]	4.8 ± 0.1	5.02 ± 0.05	0.51 ± 0.04	3.3 ± 0.2	2.08 ± 0.06	(1.25 ± 0.06)e − 6
CV[a]	3%	1%	8%	5%	3%	5%

[a]The mean and CV% were calculated based on the unexpired lots #2–6

Table II Inter-lot results of PPS quality attributes

Wang K et al. The AAPS Journal (2023) 25:50 https://doi.org/10.1208/s12248-023-00815-4

Summary

- PPS, a semi-synthetic, heparin-like polysaccharide
- Totality of Evidence for API sameness
- Structural signatures

Resources

- Sameness Evaluations in an ANDA – Active Ingredients (November 2022)
- Guidance for industry Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA. (October 2022)
- Controlled Correspondence Related to Generic Drug Development Guidance for Industry (March 2024)
- Product Specific Guidance (PSG) on Pentosan Polysulfate Sodium (May 2021)
- Wang K et al. *The AAPS Journal* (2023) 25:50, DOI: 10.1208/s12248-023-00815-4, A precise qNMR method is used for the rapid quantification of the acetylation degree and the unsaturated aldehyde and pyridinium complex in the reducing end.
- Alekseeva A et al. *Carbohydrate Polymers*. 2020; 234:115913, DOI: 10.1016/j.carbpol.2020.115913, In-depth structural characterization of pentosan polysulfate sodium complex drug using orthogonal analytical tools.
- Lenhart D et al. *Carbohydrate Polymers* 2023, *319*, 121201, DOI: 10.1016/j.carbpol.2023.121201, Chemical and biological differences between original and mimetic pentosan polysulfates.

Thank you!

Comparative Physicochemical Characterization of Iron Products

Dahui Liu, PhD
Senior Pharmaceutical Quality Assessor
Division IV, Office of Pharmaceutical Quality Assessment I/OPQ
CDER | US FDA

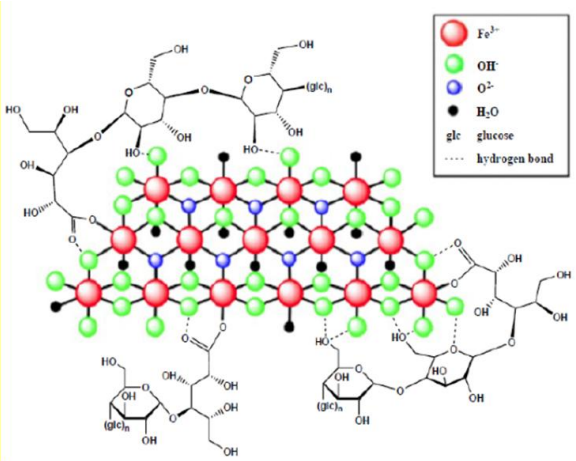
Advancing Generic Drug Development Workshop: Translating Science to Approval
October 7-8, 2025

Outline

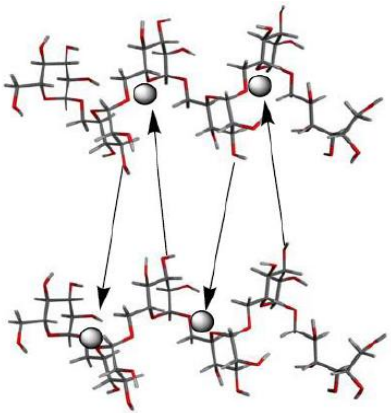
- Iron colloid products overview
- Physicochemical characterization
- Case studies
- Closing thoughts

Iron Colloid Products

- Iron colloid drug products are injection dosage forms widely used for the treatment of iron deficiency anemia. They are nano-size complexes comprised of polynuclear cores and carbohydrates.
- The carbohydrates surrounding the iron core facilitate the dispersion of the nano colloidal particles in the aqueous formulation, and upon dosing, minimize the release of iron directly to the system, thus reducing toxicity.



Ferric Carboxymaltose



Ferric Derisomaltose

Iron Colloid Products

RLD	Labeled Non-Proprietary Name	Approval Date	PSG	Approved Generics
INFeD NDA 17441	Iron Dextran	4/29/1974	2016, Revised 2022	0
Ferrlecit NDA 20955	Sodium Ferric Gluconate	2/18/1999	2013, Revised 2022	1
Venofer NDA 21135	Iron Sucrose	11/6/2000	2012, Revised 2013, 2021, 2025	3
Feraheme NDA 22180	Ferumoxytol	6/30/2009	2012, Revised 2023, 2024	1
Injectafer NDA 203565	Ferric Carboxymaltose	7/25/2013	2016, Revised 2024	0
Monoferric NDA 208171	Ferric Derisomaltose	1/16/2020	2024	0

Composition of Iron Carbohydrate Complexes

Product Name	Carbohydrate	Iron State	Strength (mg Fe/mL)	MDD* (mg Fe)	pH*
Sodium ferric gluconate	Gluconate, sucrose	Fe (III)	12.5	125	7.7-9.7
Iron Sucrose	Sucrose	Fe (III)	20	400	10.5-11.1
Ferric derisomaltose	Derisomaltose	Fe (III)	100	1000	5.0-7.0
Iron dextran	Low molecular weight dextran	Fe (III)	50	100	4.5-7.0
Ferumoxytol	Polyglucose sorbitol carboxymethylether maltose	Fe (III)/Fe (II)	30	510	6-8
Ferric carboxymaltose	Carboxymaltose	Fe (III)	50	1000	5.0-7.0

*Per RLD labeling

PSG Recommendations

The test product should be qualitatively **(Q1)** and quantitatively **(Q2)** the same as the reference listed drug (RLD).

- **Two BE Studies:** *In vivo* and *in vitro*
- **Special Considerations:**
  - Demonstrate **comparable physicochemical properties** by characterization of at least 3 batches of the test product and reference standard (RS) product, including characterizations of drug product, iron core, carbohydrate, interaction of iron core and carbohydrate, and labile iron.
  - Test batch requirement: Manufactured using a process reflective of the proposed commercial scale manufacturing process. At least one test batch should be produced by the commercial scale process and used in the *in vitro* and *in vivo* bioequivalence study.

Complexity and Challenges

- The product consists of colloid particles, free iron, unbound carbohydrate, and other small molecular weight species.
- The nanoparticles, iron core, and sometimes carbohydrate have distributions of size, structure, composition, etc. Common analytical methods for pharmaceutical products may not be appropriate for the characterization studies.
- Sample preparation may impact the physicochemical properties of the drug product.

Physicochemical Characterization Techniques

	Properties	Common Tests
Whole particle	Equivalence in stoichiometric ratios of iron, free and bound carbohydrate and other relevant components Molecular weight distribution (Mw, Mn, and Mw/Mn) Particle size Distribution Particle Morphology	Iron and carbohydrate assay, elemental analysis SEC, AUC or GPC DLS and AFM AFM
Iron core	Iron core size and morphology Crystallinity Iron environment Fe^{3+} to Fe^{2+} reduction potential and Fe (II) content Magnetic properties	TEM, XRD, SAXS Mossbauer, Raman, XRD Mossbauer, EPR, UV-Vis Polarography, Cerimetric titration VSM, SQUID
Carbohydrate	Carbohydrate composition and carbohydrate-Iron core interaction Surface properties Characterization of carbohydrate	FT-IR, thermal analysis Zeta potential NMR, SEC

P. Zou, K. Tyner, A. Raw, S. Lee, AAPS J. 2017, 1359-1376.

Considerations for Characterization Studies

- Use of orthogonal methods is recommended to reduce uncertainty in measurement of physicochemical properties.
- Pay attention to sample preparation. Sample preparation should be consistent with the purpose of the test.
- Conduct studies under a series of varied experimental conditions to enhance the differentiation ability of the test.

Case Study- Stoichiometry

- Common deficiencies:
  - The study was conducted using undialyzed sample.
  - Only assay data for iron and carbohydrate were provided.
- Recommendations:

  The test product contains—in addition to the colloid nanoparticles—unbound and loosely bound carbohydrates, low molecular weight iron complex, free iron, and other components (for example, chloride in iron sucrose). To determine the comparability of the stoichiometry of the drug product as well as the colloid nanoparticle, quantitation of each component using both as-is formulation and dialyzed (or ultrafiltered) samples are needed.

Case Study- Molecular Weight by SEC

- Common deficiencies:
  - Data used for comparative analysis were generated at different times.
  - USP monograph limits were used to justify differences between test and reference product.
- Recommendations:
  - The molecular weight determined by size exclusion chromatography (SEC) is calculated based on the standard curve, thus depends on the reference standards used and chromatography conditions. Therefore, it is important to conduct the comparative study of reference standard and test product side by side at the same time. To minimize the effect of chromatographic condition variability, we recommend you conduct the comparative molecular weight measurement of RLD and test product side by side at the same time, using the same reference standard lot and same chromatographic instrument.
  - USP limits are considered the minimum requirements for quality control and are not sufficient justification for comparability.

Case Study – Mössbauer Spectroscopy

- Common deficiencies:

  Iron-57 Mössbauer spectroscopy is a sensitive method for the characterization of the iron core crystallinity, magnetic properties, chemical environment, and core size (estimated using blocking temperature). However, the data collected may not reflect the actual properties due to sample manipulation by lyophilization.

- Recommendations:

  Due to the complex nature of the drug product, sample manipulation during the comparative characterization study could impact the actual properties of the nanoparticles. The properties of the iron core may be preserved during characterization by obtaining Mössbauer spectra using as-is drug product formulation. We recommend conducting comparative characterization of the test product and the RLD using the **as-is drug product formulation** at various temperatures. We recommend the temperature range covers the transition range where the blocking temperature (Tb) is determined

Case Study – Particle Size Distribution by DLS

- Common deficiencies:

  Particle size distribution (PSD) study by dynamic light scattering (DLS) was conducted at a single concentration.

- Recommendations:

  The concentration of the DLS samples may impact the stability of the iron colloidal particle and cause change of particle size. Since the change in particle size upon dilution can be used to assess the interactions between the carbohydrate and iron core, we recommend conducting a serial dilution PSD study to demonstrate your drug product has the same PSD trend as the RLD upon dilution.

Closing Thoughts

- The evaluation of the test product and the RLD comparability warrants a comprehensive approach with reproducible/robust results. The recommended in vitro characterization, and in vitro and in vivo bioequivalence studies in the PSGs are complimentary to each other and each is considered part of a totality of evidence approach to demonstration of sameness.
- Suitability of a characterization method, including sample preparation and testing conditions is critical for valid comparability evaluation.

Acknowledgement

OPQ - Assessment

Cameron Smith
Bing Cai
Geoff Wu

Yiwei Li
Pahala Simamora

Sherry Bai
Vasiliy Korotchenko
Tina Jiao
Jie Chen
Srinivas Ganta
Yafei Jin

OPQ-Research

Haiou Qu
Kang Chen
Charudharshini Srinivasan
Xiaoming Xu

OGD

Deyi Zhang
Darby Kozak
Yan Wang

Thank you!