



**U.S. FOOD & DRUG  
ADMINISTRATION**

# Biosimilar User Fee Act (BsUFA) III Regulatory Science Pilot Program

ANNUAL REPORT



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## Check if this report is Progress or Final Report:

Progress report

Final report

# Report Overview<sup>1</sup>

<b>Project Title:</b>	One-Pot Glycan - a chemoenzymatic method for simultaneous profiling and comparison of N- and O-glycans		
<b>Investigator:</b>	Tongzhong Ju		
<b>Organization:</b>	FDA/CDER/OPQ/OPQR		
<b>Grant No. (if applicable)</b>	N/A		
<b>Project Objective:</b>	Establish and validate a chemoenzymatic method for simultaneous profiling and comparison N-and O-glycans from purified proteins and protein drugs in a One-Pot format.		
Specific Aim(s)	Progress	Outcomes	Communication Timeline
Aim 1: Establish a method for simultaneous profiling N- and O-glycans from purified proteins and cellular proteins in a One-Pot format.	100%	<p>Established a method for simultaneous profiling N- and O-glycans from purified proteins in a One-Pot format.</p> <ol style="list-style-type: none"> <li>1. A one-pot glycomic method in a single workflow has been established.</li> <li>2. The one-pot method simultaneously profiles N- and O-glycans from glycoprotein drugs.</li> <li>3. The one-pot method measures relative abundances of permethylated N- and O-glycans, including sialylated and PK-relevant glycan determinants.</li> <li>4. The one-pot method reports the N- to O-glycan ratios in glycoproteins.</li> </ol>	<p>May 1st, 2023-Sept. 30, 2024</p> <ol style="list-style-type: none"> <li>1. <i>First OPQ ORISE Seminar (presentation)</i>: A chemoenzymatic method for simultaneous profiling of N and O-glycans in one-pot</li> <li>2. <i>FY2023 FDA Science Forum (June 13-14, 2023) (Abstract &amp; Poster)</i>: A chemoenzymatic method for simultaneous profiling of N and O-glycans in one-pot</li> <li>3. <i>Video Demo</i>: The MS&amp;I CoE Communication Working Group presented a virtual demonstration of “a new mass spectrometry method to simultaneously profile N- and O-linked glycans from protein therapeutics” and followed by an expert panel discussion on June 20<sup>th</sup>, 2024.</li> <li>4. <i>Publication</i>: Ortega-Rodriguez, U., Bettinger, J.B., Zou, G., Falkowski, V.M., Lehtimaki, M., Matthews, A.M., Biel, T.G., Pritts, J.T., Wu, W., Shen, R-F., Agarabi, C., Rao, V. A., Xie, H., and Ju, T. (2024) A chemoenzymatic method for simultaneous profiling N- and O-glycans on glycoproteins using a one-pot format. <i>Cell Reports Methods</i>, Accepted.</li> </ol>

<sup>1</sup> This section will be used by program for broader research portfolio and regulatory impact analysis by the BsUFA III steering committee.

Specific Aim(s)	Progress	Outcomes	Communication Timeline
Aim 2: Optimize and validate the method using therapeutic proteins, and biosimilars with both N- and O-glycans	30%	The one-pot method's reproducibility and intermediate precision using protein drugs have been demonstrated.	Oct. 1st, 2024-Sept. 30, 2025 1. <i>Establish a standard operating procedure (SOP)</i> 2. <i>Publish a STAR Protocol</i>

## Progress Summary

Glycosylation is often a critical quality attribute for protein drugs. The current technologies for analysis of N-glycosylation and O-glycosylation of protein drugs rely on methods to look at N-glycans and O-glycans from two separated samples using different procedures. These conventional methods are time-consuming, and the results are less informative. This project will develop and validate a one-pot method to simultaneously profile the N-, and O-glycosylation of protein drugs from the same protein drug samples. The method will provide an advanced technology for industry to have a better control strategy for the glycosylation of their protein drugs, including biosimilars, facilitate the CDER CMC reviewers' quality assessments of glycoprotein drugs and biosimilars. The specific aims and progresses are:

1. Establish a method for simultaneous profiling N-and O-glycans from purified proteins and cellular proteins in a One-Pot format. This aim has been completed, and a manuscript is accepted for publication in *Cell reports Methods*.
2. Optimize and validate the method using therapeutic proteins, and biosimilars with both N- and O-glycans, here etanercept biosimilars. A part of validation in aim 2, ~30% has been completed, and the rest is ongoing. The completed part includes the demonstration of method reproducibility and intermediate precision.

## Project Objective

1. Establish a method for simultaneous profiling and comparison of N-and O-glycans from purified proteins and glycoprotein drugs in One-Pot format.
2. Optimize and validate the method.

## Research Outcomes

The research outcomes include:

1. A one-pot glycomic method in a single workflow has been established.
2. The one-pot method simultaneously profiles N- and O-glycans from glycoprotein drugs.
3. The one-pot method measures relative abundances of permethylated N- and O-glycans, including sialylated and PK-relevant glycan determinants.
4. The one-pot method reports the N- to O-glycan ratios in glycoproteins.
5. The one-pot method demonstrates its reproducibility and intermediate precision.

## Regulatory Impact

We developed a one-pot glycomic method for simultaneous analysis of N- and O-glycans from the same sample, and in a single workflow to facilitate the comprehensive assessment of glycosylation during the biomanufacture and release of protein-based drug products, which represents a significant

advancement in the analytical assessment of N- and O-glycosylation of glycoprotein therapeutics. The regulatory impacts include:

For industry:

1. The one-pot method can be utilized for characterization and/or release testing of the glycosylation of therapeutic proteins/biosimilars to ensure the lot-to-lot consistence and bioequivalence of their biosimilars in glycosylation.
2. The available one-pot method will promote the development of biosimilar programs by facilitating the production cell line development to identify and develop the cell clones whose products have similar glycosylation profiles: N- and O-glycan ratio, levels of sialylation and PK-relevant glycan determinants.

For regulatory agency:

1. The data from the one-pot glycomic method can greatly assist the quality Assessors in assessing: a. the quality attributes of glycosylation in glycoprotein products: efficacy, PK or PD and safety; b. the lot-to-lot comparability in glycosylation of the products/biosimilars; and c. glycosylation similarity of a biosimilar to their reference product.
2. Structure-function assessments and comparisons related to O- and N-glycans of biosimilar products will be easier for Assessors to perform and correlate with other quality and safety attributes.

## Communication and Dissemination

### March 1st, 2023-September 30th, 2024

1. First OPQ ORISE Seminar, March 24, 2023 (presentation): A chemoenzymatic method for simultaneous profiling of N and O-glycans in one-pot
2. FY2023 FDA Science Forum, June 13-14, 2023 (Abstract & Poster): A chemoenzymatic method for simultaneous profiling of N and O-glycans in one-pot
3. Video Demo: The MS&I CoE Communication Working Group presented a virtual demonstration of “a new mass spectrometry method to simultaneously profile N- and O-linked glycans from protein therapeutics” and followed by an expert panel discussion on June 20<sup>th</sup>, 2024.
4. Publication: Ortega-Rodriguez, U., Bettinger, J.B., Zou, G., Falkowski, V.M., Lehtimaki, M., Matthews A.M., Biel, T.G., Pritts J.T., Wu W., Shen, R-F., Agarabi, C., Rao. V. A., Xie, H., and Ju, T., (2024) A chemoenzymatic method for simultaneous profiling N- and O-glycans on glycoproteins using a one-pot format. *Cell Reports Methods*, Accepted.

### October 1st, 2024-September 30th, 2025

1. Establish a standard operating procedure (SOP)
2. Publish a STAR Protocol

## Challenges

The overall timelines for each Aim of the project are being adjusted as needed.

## Next Steps

1. Continue advancing and validating the one-pot method for biosimilars with N- and O- linked glycans.
2. Establish a Standard Operating Procedure (SOP) for the one-pot method.

## Appendix: Abbreviations

Abbreviation	Definition
N-glycan	Asn- or N-linked glycan
O-glycan	Ser- and Thr- or O-linked glycan
CQA	Critical Quality Attribute
MS	Mass Spectrometry
PK	Pharmacokinetics