IN VITRO CHARACTERIZATION OF THE GENERIC PEPTIDE THERAPEUTIC: THE GLP-1R AGONIST CASE STUDY

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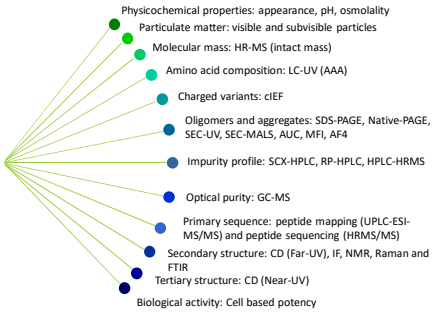
INTRODUCTION

Newer generation of antidiabetics such as glucagon-like peptide-1 receptor agonists (GLP-1RA) have shown to be an efficient adjunctive therapy in patients with diabetes type 2 who failed to respond to oral antihyperglycemic agents [1, 2]. By the 2045 it has been estimated that 700 milion people will have diabetes, chronic disease which has risen to major global health issue [3]. Generic version of GLP-1RA would decrease drug price vastly and would make newer diabetes medications widely accessible.

WORLD

2045 700 million
2030 578 million
2019 463 million

51 % increase



In order to meet regulatory requirements [4] a comprehensive analytical characterization needs to be performed and an equivalency of the generic Exenatide injection (Gx) to the innovator drug must be demonstrated. Exenatide is a 39-amino acid peptide which stimulates GLP-1R to cause insulin release [1]. In this work, the extensive comparability study, which was aimed to ensure quality, safety and efficacy of the generic peptide product similar to the Reference Listed Drug (RLD) Byetta®, is described. *In vitro* characterization of the generic drug included various analyses of physicochemical properties such as identity, structure, self-association and aggregation studies, purity and peptide content determination, whereby different orthogonal analytical techniques were employed. Most of the analytical methods were developed and optimized in-house. The comparison study was performed on different batches of the Exenatide injection and RLD samples.

1 Exenatide primary structure

HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-[NH2]

- Impact on safety and efficacy
- Sameness of Gx and RLD product by peptide mapping (UPLC-HRMS), peptide sequencing (HRMS/MS) and intact mass (MS)

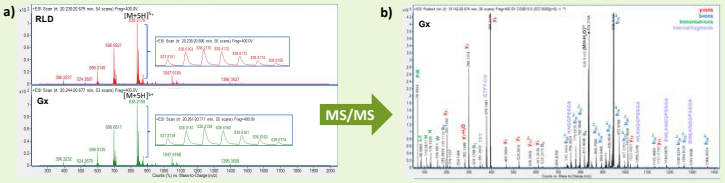


Figure 1. Intact mass and peptide sequencing by ESI-MS/MS: a) Full scale MS spectra of the RLD Byetta® and the Exenatide injection (Gx) with assigned $[M+5H]^{5+}$ ions and zoomed-in isotopic distribution; b) Assigned MS/MS spectra of the $[M+5H]^{5+}$ (m/z 838) of Gx sample

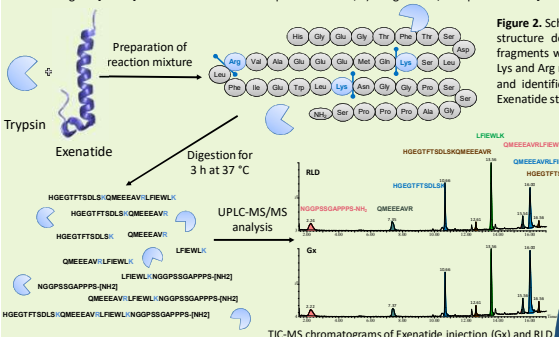


Figure 2. Schematic overview of Exenatide primary structure determination by peptide mapping – fragments were produced by trypsin hydrolysis of Lys and Arg residues, separated by UPLC technique and identified by MS/MS. For illustration of the Exenatide structure PDB ID 1JRJ was used [5].

Table 1. Exenatide process related (API) and degradation impurities analyzed by orthogonal techniques – RP-HPLC and SCX-HPLC. Impurities are marked in chromatograms in the Figure 3.

Impurity	Modification
D, E, G, H, N, Q, S	process (API)
A, C	deamidation
I	deamination
B	oxidation
F, G, K, M	truncation
O	acetylation
J, L	dehydration
R, T	isomerization
P	spinacine formation

Impurities O, P, R, S and T co-eluted with main Exenatide peak in RP-HPLC (Figure 3 a) and were resolved from the main peak in SCX-HPLC (Figure 3 b) chromatograms.

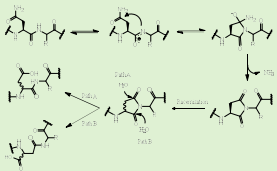


Figure 4. Mechanism of asparagine deamidation – example of common degradation impurity formation

2 Peptide - related impurities

- Impact on safety and efficacy
- Risk of immunogenicity
- Sensitive, high-resolution (HPLC-HRMS) and orthogonal analytical chromatographic techniques e.g. RP, IEX, HILIC, HIC…

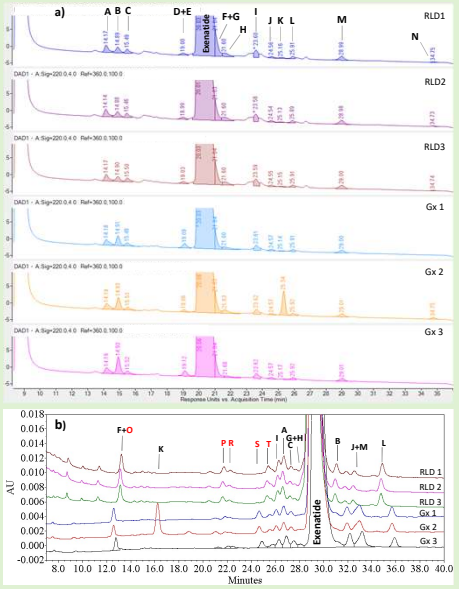


Figure 3. Exenatide impurity profile comparison of the Exenatide injection (Gx) and the RLD Byetta® samples by the means of a) RP-HPLC and b) SCX-HPLC methods; different selectivity achieved by orthogonal chromatographic techniques – red marked impurities co-eluted with the main Exenatide peak in RP-HPLC (Figure a) and were separated in SCX-HPLC (Figure b) chromatograms

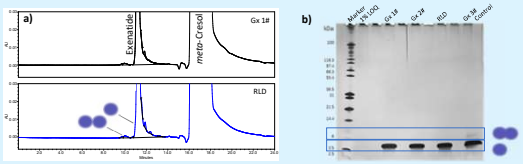
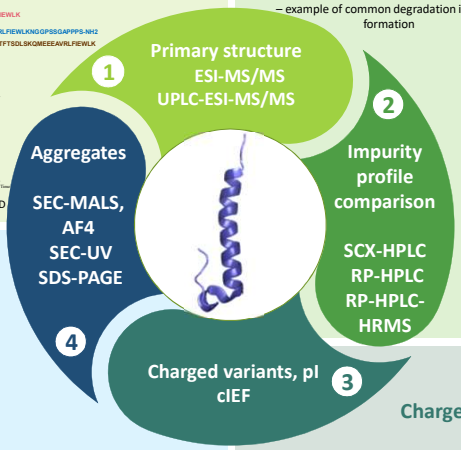


Figure 6. Irreversible aggregates determination by orthogonal techniques: a) SEC-UV chromatogram of the Exenatide injection (Gx) 1# and RLD Byetta® samples; b) SDS-PAGE comparison of different Gx batches and RLD



Aggregates

- Impact on safety and efficacy
- Enhance immune response
- Irreversible (SEC-UV, SDS-PAGE) and reversible (AF4, SEC-MALS)
- Different methods required for a wide range of aggregates
- Sensitive, orthogonal methods to assure accuracy and robustness

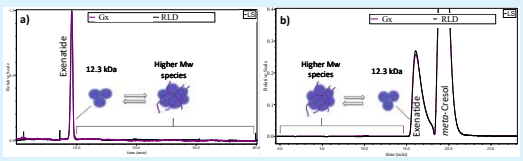


Figure 7. Self-association and reversible aggregates comparison of the Exenatide injection (Gx) and the RLD Byetta® by orthogonal techniques a) AF4 and b) SEC-MALS

4

Charge heterogeneity

- Impact on efficacy
- Isoelectric point (pI) determination and charge heterogeneity comparison
- Bioinformatical and literature data screen and comparison with experimental data
- High resolution separation of peptide charge variants by cIEF

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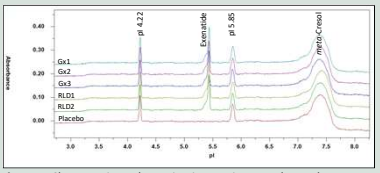

Figure 5. Charge variants determination: native cIEF electropherograms of the Exenatide injection (Gx) and the RLD Byetta® samples

Site Name	Owned by	pI Value
Protein Isoelectric Point Calculator	Lukasz P. Kozlowski	4.55
Protein Calculator	The Scripps Research Institute	4.70
PepCalc	Innovagen AB	4.77
Karger Kidney and Blood Pressure Research	Yi Zhang et al.	4.86
Peptide Calculator	Bachem	4.90
Biomaterials	Jae Y. Kim et al.	4.96

Table 2. List of pI databases and literature sources with theoretical pI values

CONCLUSION

The performed characterization of different batches of the generic Exenatide injection and the RLD Byetta® proved the same primary structure, aggregation propensity and charge heterogeneity. Moreover, similar impurity profiles of Exenatide injection and RLD were confirmed. For impurities which were not detected in RLD batches or levels were higher in generic Exenatide drug, the immunogenicity risk assessment was conducted. It was shown that generic Exenatide injection does not contain impurities which induce greater or distinct stimulation of innate or functional immunoresponse than the RLD Byetta®. Furthermore, comparability of Exenatide's attributes such as physicochemical properties, identity, secondary and tertiary structure, and biological activity were evaluated. It is demonstrated that the generic Exenatide injection drug shows highly similar quality attributes related to safety and efficacy, including immunogenicity, and meets the same standards as the innovator drug.

LITERATURE

[1] Cvetkovic R.S. and Plosker G.L., Drugs 67 (2007) 935-954
[2] Cai Y et al., Drug Des Devel Ther. 7 (2013) 963-970
[3] IDF Diabetes atlas, Ninth edition 2019, International Diabetes Federation (www.diabetesatlas.org)
[4] ANDAs for Certain Highly Purified Synthetic Peptide Drug Products that Refer to Listed Drugs of rDNA Origin, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), May 2021
[5] PDB ID: 1JRJ; Jonathan W. Neidigh et al. Biochem. 40 (2001) 13188–13200

međunarodni znanstveno-stručni skup
19. Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
21. – 23. rujna 2022. | Vukovar, Hrvatska