Research Article

Comparative Bioavailability Study of Rybelsus (Semaglutide) 3 mg Tablets of Novo Nordisk *Versus* Ozempic (Semaglutide) 2 mg/1.5 ml (1.34 mg/ml) Solution for Injection of Novo Nordisk under Fasting Condition in Healthy Subjects

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ABSTRACT

Background: The human GLP-1 receptor agonist, semaglutide, functions by binding to albumin, which is facilitated by modifying position 26 lysine with a hydrophilic spacer and a C18 fatty di-acid. In this study, Novo Nordisk's Ozempic (Semaglutide/1.5 mL (1.34 mg/mL) solution for injection and Rybelsus (Semaglutide) 14 mg tablets) were compared to determine the pharmacokinetic parameters of Semaglutide after oral and subcutaneous administration in healthy subjects. Through the Flash Glucose Monitoring System with a sensor, we evaluated the pharmacodynamic response of semaglutide the absorption of glucose into the blood when the drug was administered in two different routes, using Novo Nordisk's Semaglutide 3 mg tablets in healthy subjects. To evaluate pharmacokinetics of Semaglutide injection and tablet and to assess the applicability of an LCMS/MS quantification method.

Materials and methods: Dose and mode of administration, batch, expiry date, Ozempic (Semaglutide) 2 mg/1.5 mL (1.34 mg/mL) solution for injection, 0.37 mL × 1.34 mg (0.5 mg), subcutaneous injection in sitting posture under fasting condition, MP5E309, July, 2025. Dose and mode of administration, lot, expiry date, Rybelsus (Semaglutide) 3 mg tablets, 01 × 3 mg, oral with 240 ± 02 mL of water in sitting posture under fasting condition, MS6GG22, Feb, 2024. The 90% confidence intervals were evaluated for comparison and to evaluate the absolute bioavailability of C_{max} and AUC_{0-t}. The 90% confidence intervals for C_{max} and AUC_{0-t} were 14.78%-58.01% and 4.51%-17.65% respectively.

Conclusion: The study was conducted as a proof-of-concept study to evaluate the pharmacokinetics of semaglutide when administered-subcutaneously and when administered as a tablet through the oral route. The pharmacokinetics obtained are similar to the reported values and when compared, the absolute bioavailability is about 1%-2% between these two products.

Keywords: Semaglutide; Bioavailability; Pharmacokinetic; Healthy subjects

INTRODUCTION

Semaglutide is a human GLP-1 receptor agonist (or GLP-1 analog). The main protraction mechanism of semaglutide is albumin binding, facilitated by modification of position 26 lysine with a hydrophilic spacer and a C18 fatty di-acid [1-3].

Furthermore, semaglutide is modified in position 8 to provide stabilization against degradation by the enzyme dipeptidyl-peptidase 4 (DPP-4). A minor modification was made in position 34 to ensure the attachment of only one fatty di-acid [4,5]. Each Ozempic solution for injection contains 2 mg/1.5 mL (1.34 mg/mL) of Semaglutide, and each Rybelsus tablet contain 14 mg of Semaglutide. Its molecular formula is $C_{187}H_{291}N_{45}O_{59}$, and it has a molecular weight of 4113.58 g/mol.

MATERIALS AND METHODS

The study design was an open-label, balanced, randomized, single dose, two treatments, single period, parallel, comparative bioequivalence study under fasting condition. The study was conducted on six male subjects in the age group of 22 to 42 years who met the study eligibility criteria. The study was conducted with subcutaneous injection and orally administered tablets [6]. From the six subjects, three subjects received subcutaneous injection and the other three received oral tablets. Blood samples were collected up to 96 hours and 144 hours post-dose, respectively, for subjects dosed with tablet and injection. These samples were used for measurement of pharmacokinetic parameters of both the products. The pharmacodynamic response of the drug when administered in two different routes was also evaluated. Safety evaluation was done by assessing clinical examinations, vital signs assessments, clinical laboratory parameters, and monitoring the subject's well-being, symptoms and signs for adverse events. A validated LC-MS/MS method was used to determine the plasma concentrations of semaglutide [7].

Volunteers

A total of six subjects were selected and allowed to participate in the study.

Inclusion criteria for this study were:

a) Healthy male literate volunteers of 18 to 45 years (both years inclusive) and weight >50 kg.

b) Healthy volunteers within acceptable body mass index as deemed by the investigator.

c) Healthy volunteers as evaluated by medical history, vitals and general clinical examination.

d) Normal or clinically insignificant biochemical, hematological, urine and serology parameters.

e) Normal or clinically insignificant ECG.

f) Negative urine test for drugs of abuse, alcohol breath analysis.

g) Volunteers who are willing to use acceptable methods of contraception.

h) Volunteers who can give written informed consent and communicate effectively [8,9].

Exclusion criteria for this study were:

a) History of any major surgical procedure in the past 3 months.

b) History of any clinically significant cardiac, gastrointestinal, respiratory, hepatic, renal, endocrine, neurological, metabolic, psychiatric and hematological disorders.

c) History of chronic alcoholism/chronic smoking or drug abuse.

d) Volunteers with known hypersensitivity to semaglutide or any of the excipients.

e) History of consumption of tobacco-containing products within 48 hours prior to the proposed time of dosing.

f) Volunteers who are positive for hepatitis B surface antigen, anti-hepatitis C antibody, treponemal antibodies and Human Immunodeficiency Virus (HIV 1 and 2) antibodies.

g) Volunteers with a history of intake of drugs or any prescription drug or Over-the-Counter (OTC) drugs/vaccines (including COVID-19 vaccines) within 14 days or a significant duration as deemed by the investigator that potentially modify the kinetics/dynamics of semaglutide or any other medication judged to be clinically significant by the investigator.

h) History of consumption of grapefruit and/or its products within 10 days prior to the start of study.

i) Volunteer who had participated in any other clinical study or who had bled during the last 3 months before check-in.

j) History of consumption of one or more of the below, 48 hours prior to dosing: Xanthine containing food or drinks such as cola, chocolate, coffee or tea, citrus fruits or items (lime, lemon, and orange), alcohol and any other food or beverage known to have interactions as deemed by the investigator [10,11].

Study design

An open-label, balanced, randomized, single dose, two treatments, single period, parallel, comparative bioequivalence study of Rybelsus (Semaglutide) 3 mg tablets of Novo Nordisk and Ozempic (Semaglutide) 2 mg/1.5 mL (1.34 mg/mL) solution for injection of Novo Nordisk in healthy, adult, human male subjects under fasting conditions.

Study subjects received either an injection or a tablet per the randomization schedule (Figure 1).
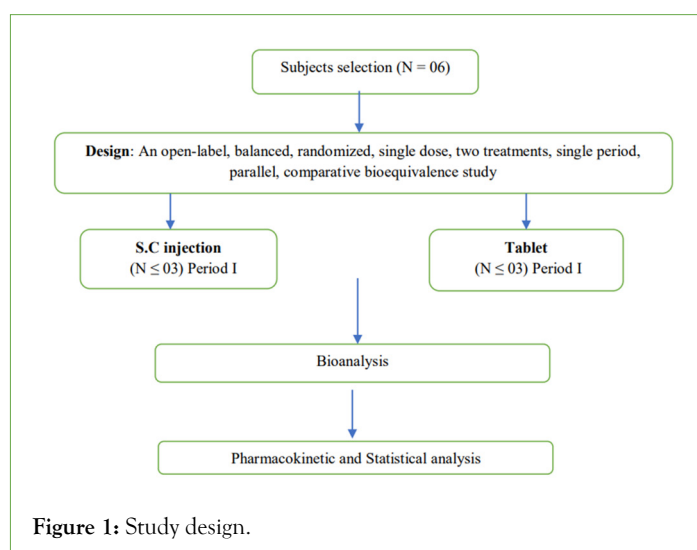

Figure 1: Study design.

Drug administration

After an overnight fasting of 8 hours, the subjects were administered a single oral dose of either of the products. The injection was administered to three subjects as directed in the product information. The tablet was administered with 240 mL of water. Compliance with dosing following drug administration was assessed by examination of the oral cavity. Drug dosing details were recorded in the individual case report forms. The principal investigator and/or sub-investigator/physician were present throughout the conduct of the study. All the subjects remained in sitting posture for 2 hours after dosing and were permitted to walk for short intervals of time for reasons such as but not limited to the following: Natural exigencies, blood sampling and vitals measurement activity. The subjects received standard food approximately at 04.00, 08.00 and 12.00 hours post-dose with time flexibility of more than 15 minutes.

Blood sampling

Tablet: A total of 22 blood samples of 5 ml each at 00.00 (pre-dose), 00.17, 00.33, 00.50, 00.75, 01.00, 01.50, 02.00, 02.50, 03.00, 04.00, 05.00, 06.00, 07.00, 08.00, 10.00, 12.00, 24.00, 34.00, 48.00, 72.00, and 96.00-hours post-dose, were collected for measurement of pharmacokinetic parameters in each period.

Injection: A total of 21 blood samples of 5 ml each at 00.00 (pre-dose), 02.00, 04.00, 08.00, 12.00, 24.00, 28.00, 32.00, 36.00, 40.00, 44.00, 48.00, 52.00, 56.00, 60.00, 64.00, 72.00, 84.00, 96.00, 120.00 and 144.00-hours post-dose were collected for measurement of pharmacokinetic parameters in each period. The pre-dose samples were collected two hours before the drug administration.

The post-dosage in-house samples were collected within 2 min of the scheduled time, and the ambulatory samples were collected within ± 1 hours from the scheduled time. Any delay from these was recorded as protocol deviation. The samples were collected from intravenous cannulas or by direct vein puncture into pre-labeled K_3EDTA vacutainers. The samples were then centrifuged at 3500 rpm for 10 min at 4°C. The plasma was separated into two aliquots: 1 ml in the first aliquot and remaining in the second aliquot. The K_3EDTA vacutainers and polypropylene tubes were pre-labeled with the subject number, study number, study period, time point and aliquot number. The samples were stored at -70°C in an ultra-low temperature freezer. The plasma samples were quantified using validated bioanalytical methods.

Glucose monitoring

A flash glucose monitoring system was used to monitor the glucose levels through a sensor. A sensor was fixed to the back of the arm and the glucose levels were monitored for 3 days.

Analytical method

Liquid-liquid extraction was chosen for sample preparation to extract the analyte of semaglutide. A validated LC-MS/MS bioanalytical method was used for the estimation of semaglutide in plasma. Bioanalytical method validation was done as per the guidance. All samples from one subject were analyzed with the same single standard curve. Sample concentration above the upper limit of the standard curve from the validated range was analyzed by diluting the sample with a drug-free biological matrix and assaying.

Pharmacokinetic parameters and statistical analysis

The analysis of variance (ANOVA) was performed on the Ln-transformed data of C_{max} and AUC_{0-t} using PROC GLM of SAS®

(version 9.4) software.

The ANOVA was performed on the Ln-transformed C_{max} and AUC_{0-t} parameters. The least-squares mean ratios, 90% CI for C_{max} and AUC_{0-t} of Semaglutide for both the products.

Safety analysis

Safety was assessed *via* continuous health monitoring and scheduled recording of safety measurements throughout the study. Staff monitored and recorded the pulse rate, oral temperature, blood pressure and subjects' well-being during check-in at 00.00 (pre-dose), 03.00, 09.00, 32.00, 54.00, 74.00 and 95.00-hours post-dose, post-study and at the discretion of the clinical staff. Temperature will be recorded during check-in, before check-out (95.00 hours post-dose), post-study, along with other vital parameters, and at the discretion of clinical staff. The pre-dose (00.00 hours) vital parameters were recorded within two hours before drug administration and the post-dose vitals with a flexibility of ± 30 min of each time point.

General examination and systemic examination of subjects were performed during check-in of each period by the physician/investigator/sub-investigator to ensure safety. Subjects were instructed to inform clinic personnel of any untoward medical symptoms and/or events that arose during the course of the study.

The principal investigator/sub-investigator/study physician evaluated the relationship of all adverse events to the study drugs. The adverse events were graded as mild, moderate, or severe as per standard operating procedure. The principal investigator/sub-investigator also evaluated the subjects for subsequent dosing. Post-clinical laboratory tests (hematology and serum chemistry) and post-study general and systemic examination, including vital sign measurements (blood pressure, pulse rate and temperature), were performed.

RESULTS

Pharmacokinetics and statistics

In the present study, six subjects participated, all subjects completed the study. The concentrations of all the subjects were subjected to pharmacokinetic and statistical analysis. The plasma concentration *vs.* time curve of both the products is presented in (Figures 2 and 3). The geometric mean ratios, 90% CI, and power of variation of both the products for Ln transformed pharmacokinetic parameters C_{max} and AUC_{0-t} for semaglutide are presented in Tables 1 and 2 includes the pharmacokinetics of Semaglutide after oral and subcutaneous administration. The glucose levels of all subjects at various times of a day were plotted and presented in (Figure 4).
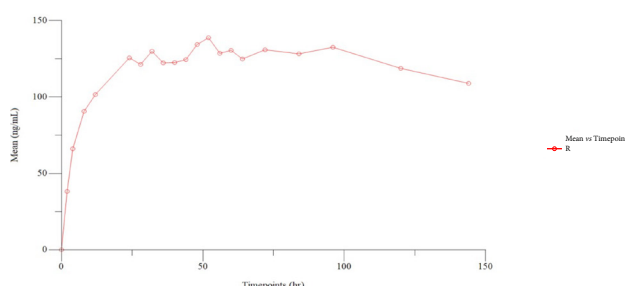
Figure 2: Bioavailability curve (mean plasma concentration *vs.* time) of Semaglutide S.C. injection.
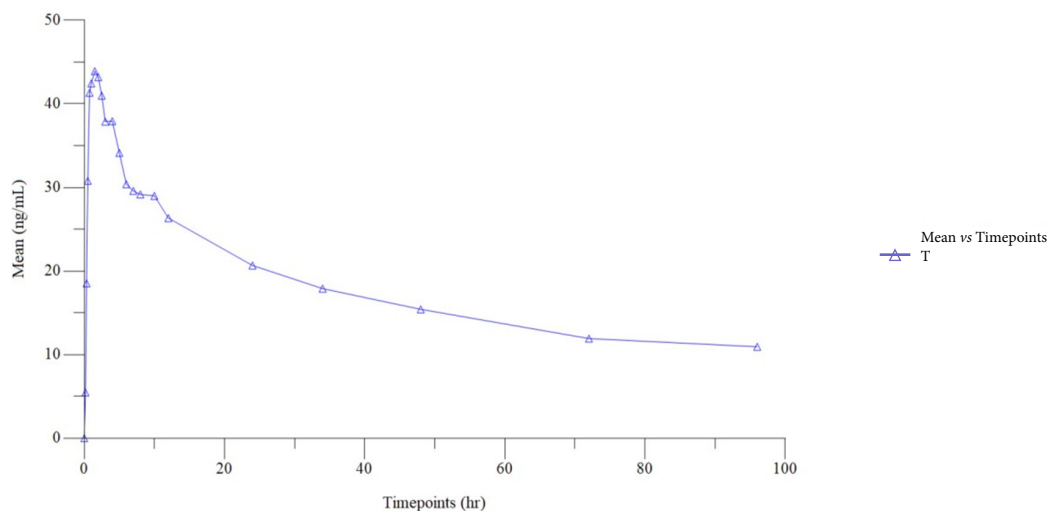
Figure 3: Bioavailability curve (Mean plasma concentration *vs.* time) of Semaglutide tablets.

Table 1: The geometric mean ratios, 90% CIs, power and inter-subject coefficient of variation of tablet and injection for Ln transformed pharmacokinetic parameters C_{max} and AUC_{0-t} for semaglutide are presented below.

Pharmacokinetic parameters	Test geometric mean	Reference geometric mean	Test/Reference ratio	90% Confidence interval for test *vs.* reference	Power of ANOVA	Inter subject cv
LN_C_{max}	41.5014	141.7167	29.28	14.78%-58.01%	0.1122	40.83
LN_AUCT	1531.6591	17174.648	8.92	4.51%-17.65%	0.1123	40.77

Table 2: Summary of pharmacokinetic parameters for semaglutide.

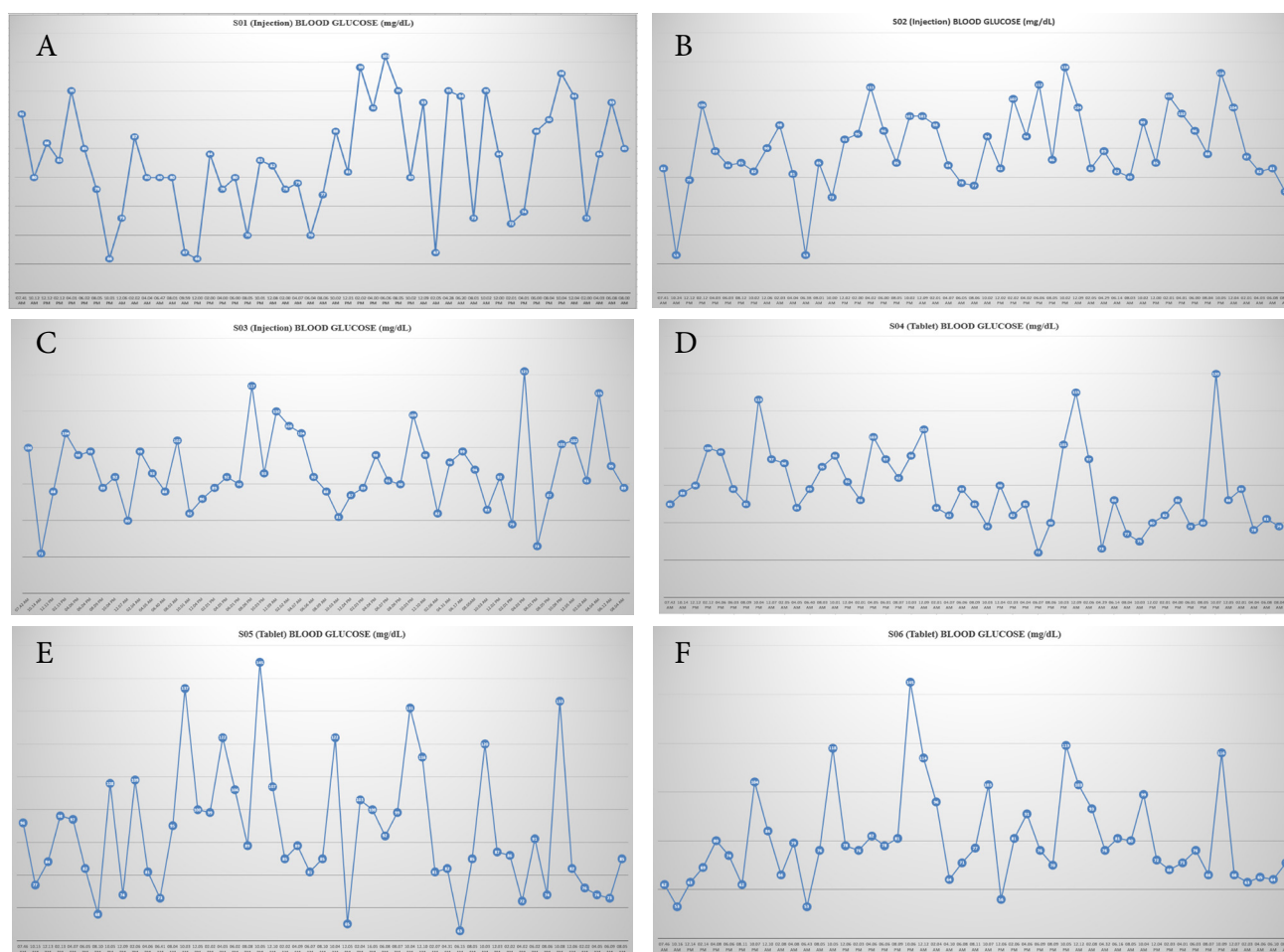| Parameter	Ozempic (Semaglutide) (Mean ± SD)	Rybelsus (Semaglutide) (Mean ± SD)
C_{max} (ng/mL)	141.942 ± 9.835	46.236 ± 27.619
AUC_{0-t} (ng.hr/mL)	17208.388 ± 1306.624	1698.962 ± 969.003
$AUC_{0-\infty}$ (ng.hr/mL)	63591.111 ± 27007.229	2593.373 ± 1421.879
t_{max} (hr)	60.00 (48.00-96.00)	0.75 (0.50-2.00)
K_{el} (hr^{-1})	0.002 ± 0.001	0.012 ± 0.001
$T_{1/2}$ (hr)	298.577 ± 110.130	57.588 ± 4.510
AUC_%Extrap (%)	71.673 ± 10.053	34.733 ± 3.031

Figure 4: A-F) The glucose levels of all subjects at various times of a day.

Brief summary of adverse events

Safety was evaluated throughout the study and there were no adverse events. No serious adverse events were reported in this study. Thus, both the test and reference products were well tolerated.

DISCUSSION

This study was conducted as a proof-of-concept study to understand the pharmacokinetics of the subcutaneous injection and the orally administered tablet as a single dose to healthy subjects. Considering the long half-life of the drug, and to assess the suitability of an LCMS/MS application to quantify the drug levels parallel design was adopted.

Blood samples were collected at pre-specified points after dosing to obtain a pharmacokinetic profile of both the products. Continuous glucose monitoring was also performed for all the subjects to observe the effect of the drug on glucose absorption and metabolism. This continuous glucose monitoring was performed using a flash glucose monitoring system through a sensor attached at the back of the upper arm. This enabled monitoring of glucose continuously for 4 days.

The study results obtained were satisfactory, and the data obtained provides an overview of the pharmacokinetics and pharmacodynamic properties of both drugs after a single dose administration. Though the study was not intended to evaluate the bioequivalence of both the products, an Analysis of Variance

(ANOVA) of C_{max} and AUC parameters were evaluated. As reported [4], the bioavailability of the orally administered tablet is about 1% of the injection administered subcutaneously, despite the dose being only about 16% of the tablet's dose. The effect on the glucose levels is evidently seen for both products. No appreciable spikes of glucose concentration were observed for 3 days after administration for both products.

CONCLUSION

The pharmacokinetics of semaglutide administered subcutaneously and orally were measured with the application of a suitable LCMS/MS method. Though a relatively low dose was administered orally, semaglutide could be quantified in all the samples. The interchangeability between two dosage forms could not be studied considering limited sample size. This study will be helpful in designing further studies in a large sample size with different objectives.

INFORMED CONSENT

The protocol and Informed Consent Forms (ICFs) were reviewed and approved by an independent ethics committee. Independent Ethics Committee, Chennai (Ethics Committee re-registration No. ECR/111/Indt/TN/2013/RR-19 issued under Rule 122D of the Drugs and Cosmetics Rules, Ministry of Health and Family Welfare, Directorate General of Health Services, Office of the Drugs Controller General (India), Central Drugs Standard Control

Organization), before study initiation. The study-informed consent documents were distributed to all the eligible subjects. The consent documents were read and explained by the staff. Subjects were given adequate time to read and understand the consent documents. Illiterate volunteers were explained vocally by the investigator in their vernacular language. Individual counseling was then given to the willing volunteers by the investigator in private, and any questions and concerns were addressed before obtaining consent. Before the initiation of the study, written informed consent was acquired from each of them, and the study was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and national regulatory requirements.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

FUNDING STATEMENT

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