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Bioequivalence of Two Different Nicotine Chewing Gum Formulations of Two Different Strengths (2 mg and 4 mg) in Indian Healthy Adult Human Male Smoker Subjects

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Abstract

Nicotine Mint Flavored Chewing Gums are used to aid smokers wishing to quit or reduce prior to quitting. The aim of these two studies was to determine the bioequivalence of two test and two reference formulations of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gum. Both of these two studies were single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design conducted in two different sets of 54 healthy adult Indian male subjects each under fasting conditions with a washout period of 8 days (for 2 mg study) and 9 days (for 4 mg study). Study formulations were administered after a 10-hour overnight fast. Blood samples for pharmacokinetic profiling were taken post-dose up to 24 hours. Safety was evaluated through the assessment of adverse events, and laboratory tests. Plasma concentrations of Nicotine were determined with a validated LC-MS/MS method. Bioequivalence between the products was determined by calculating 90% confidence intervals (90% CI) for the ratio of C_{max} and AUC_{0-t} values for the test and reference products, using logarithmic transformed data. The 90% CI of Nicotine were 93.71-113.45, 89.95-112.68 and 92.90-115.98 for $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty}$ respectively for Nicotine 2 mg Mint Flavored Chewing Gum study. The 90% CI of Nicotine were 88.68-102.76, 91.48-109.00 and 91.14-111.66 for $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty}$ respectively for Nicotine 4 mg Mint Flavored Chewing Gum study. Since the 90% CI for C_{max} and AUC_{0-t} were within the 80-125% interval, it was concluded that the two test and two reference formulations of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gum are bioequivalent in their rate and extent of absorption.

Keywords: Nicotine 2 mg; Nicotine 4 mg; Chewing gum; Smoking cessation; Nicotine replacement therapy; Bioequivalence; Pharmacokinetics

Abbreviations: AUC_{0-t}: Area Under the Plasma Concentration versus Time Curve from Time 0 to Time t; AUC_{0-inf}: Area Under the Plasma Concentration versus Time Curve from Time 0 Extrapolated to Infinity; C_{max}: Maximum Plasma Concentration; CI: Confidence Interval; CV: Coefficient of Variation; °C: Degree Centigrade; cm: Centimeter; ≥: Greater than or Equal to; hr(s): Hour(s); K_e: Elimination Rate Constant; kg(s): Kilogram(s); LC-MS/MS: Liquid Chromatography–Mass Spectroscopy/Mass Spectroscopy; ≤: Less than or Equal to; LOQ: Lower Limit of Quantification; min(s): Minute(s); mm: Millimeter; m: Meter; mM: Millimol; µl: Microliter; ng/mL: Nano gram/Milliliter; %: Percent; ppm: Parts per Million; PK: Pharmacokinetic; rpm: Rotations per Minute; SAS: Statistical Analysis Software; T_{max}: Time to reach C_{max}; $t_{1/2}$: Elimination Half-Life; yr.(s): Year(s)

Introduction

Smoking is the leading preventable cause of illness and premature death worldwide and increases the risk for cancer, cardiovascular and lung diseases, among others. Some medications have been proven to help people to quit, with three licensed for this purpose in Europe and the USA: nicotine replacement therapy (NRT), bupropion, and varenicline. Cytisine (a treatment pharmacologically similar to varenicline) is also licensed for use in Russia and some of the former socialist economy countries. Other therapies, including nortriptyline, have also been tested for effectiveness [1-5]. Accordingly, smoking cessation provides immediate and lasting benefits to public health [6-8]. However, relatively few smokers succeed in quitting each year [9]. Nicotine replacement therapy (NRT) helps smokers quit by providing nicotine at levels usually lower than those obtained through smoking and without the toxins contained in tobacco smoke. NRT can reduce the craving for nicotine and the nicotine withdrawal symptoms which might otherwise jeopardize the smoking cessation efforts [10].

Currently several dosage forms are available for Nicotine replacement therapy apart from chewing gum such as- patches, nasal sprays, inhalers, sublingual tablets, lozenges, and electronic cigarettes.

The strength of gum to be used will depend on the smoking habits of the individual. In general, if the patient smokes 20 or less cigarettes a day, 2 mg nicotine gum is indicated. If more than 20 cigarettes per day are smoked, 4 mg nicotine gum will be needed to meet the withdrawal of the high serum nicotine levels from heavy smoking [11].

Two new generic formulations of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gums were developed having the same composition as innovator brand, (NICORETTE® Freshmint) 2 mg and 4 mg nicotine, as nicotine resinate, mint flavored chewing gums of MCNEIL CONSUMER HEALTHCARE GMBH. A single dose of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gum has been evaluated in these two studies. The pharmacokinetics of Nicotine were evaluated in 54 healthy male subjects in both the two studies. The aim of these two studies was to determine the Bioequivalence and to compare the pharmacokinetics of two test and two reference formulations of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gum.

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Materials and Methods

Subjects

In each study, a total of 54 Asian Indian healthy adult human male smoker subjects who were part of Sitec healthy volunteer pool representing the general population were enrolled. Male subjects (light smokers) between 18-45 years of age having body mass index ≥ 18.5 kg/m^2 and ≤ 30.00 kg/m^2, who smoke ≥ 5 cigarettes per day regularly since last three months and who had exhaled carbon monoxide (CO) levels ≥ 10 ppm at the time of screening were eligible for participation in the study. The demographics of all 54 recruited subjects of both the studies (2 mg and 4 mg) are summarized in Table 1.

The subjects were screened within 21 days prior to study enrolment. The screening procedure included general history (including previous participation in clinical study/blood donation, alcohol and tobacco consumption); demographic data, including name, sex, race, age, body weight (kg), height (m); medical history, physical examination, vital signs measurement, a 12-lead electrocardiogram (ECG), hematology, biochemistry, urine analysis, testing for HIV I and II; hepatitis B and C.

Oral cavity examination, exhaled CO level and history or presence of dentures or any dental work (including missing molars), history of any form of oral and/or pharyngeal inflammation and any form of oral lesions and/or gum disease or tempero-mandibular joint dysfunction, tongue piercings and suffering from xerostomia (dry mouth) were examined. Subjects were judged to be healthy based on acceptable physical examination, and clinical laboratory test results. The clinical investigator reviewed the screening data and performed the physical examinations.

Informed consent and ethical approval

The protocol and informed consent forms (ICFs) were reviewed and approved prior to study initiation by an independent ethics committee. All the subjects were informed about the purpose, nature, procedure, duration, anticipated risks and discomfort of the study in the language they understand. Adequate time was given to read and understand the ICF and a written informed consent was obtained from each one of them prior to study initiation. This clinical trial was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines and national regulatory requirements [12-15]. The studies were conducted from October to November, 2012 (nicotine 2 mg chewing gum) and September to November, 2012 (nicotine 4 mg chewing gum).

Study design

Both the studies were open label, randomized, single-dose, two-treatment, two sequence, two-period, cross-over design, and comparative oral bioavailability studies. A single dose of two different test formulations of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gum were compared with two different reference formulations of (NICORETTE® Freshmint) 2 mg and 4 mg nicotine, as nicotine resinate, mint flavored chewing gum of MCNEIL CONSUMER HEALTHCARE GMBH.

The subjects did not consume any food and beverages containing xanthine or alcohol (48 hrs before dosing and throughout the period of sample collection), grapefruit (7 days before dosing and throughout the study), or vitamins (throughout the confinement period). Medications (including herbal and over-the-counter products) were prohibited for the 14 days preceding the study and also during the study.

On check in day, at least 36 hrs prior to each dosing, all subjects were screened for cocaine, cannabinoids, benzodiazepines, Opioids, Amphetamines, barbiturates and alcohol. Oral cavity examination and exhaled CO levels were checked. In both the studies, a total of 54 subjects who satisfied all the criteria for inclusion were admitted to the study center in the evening before dosing (Day-1). Both the studies were conducted in two batches (Batch A and Batch B). Batch A consisted of 26 subjects (subject No. 01 to 26) and batch B consisted of 28 subjects (subject No. 27 to 54). On the check-in day, subjects' belongings were thoroughly checked and they were asked to remove all outer garments and take a shower (including hair wash). Subjects wore clothing provided by Sitec for the duration of confinement.

Then, they were assigned to each treatment sequence as per the randomization scheme. All study medications were stored in a pharmacy under controlled conditions of temperature (22±3°C) and 50 to 60% relative humidity and was monitored continuously. A SAS generated randomization code was used to ensure balanced permutation of the treatments.

Drug administration

All the subjects received doses of Nicotine 2 mg or 4 mg chewing gum of test or the reference formulation on the dosing day (whichever was applicable in the corresponding study). Drug administration was standardized as follows: Test or the reference product was chewed at a rhythm set by an audible timer. The chewing rate was 40 chews per minute. After every 30 seconds, the subjects were instructed to move the gum to the other side of the mouth and to swallow their saliva on a verbal command by the dosing supervisor. The gum was chewed for 30 minutes without spitting or swallowing the gum. Subject compliance was closely monitored with 1 dosing supervisor for not more than 5 subjects. The start time and end time of dosing was recorded. One practice chewing session using a commercially available chewing gum was held on Day -1 prior to dosing in the both the period.

After dosing, chewed gum cuds were collected in labelled plastic bags with zipper and were stored at –20 ± 10°C until shipment to sponsor.

Study subjects were required to abstain from smoking for at least 36 hr prior to dosing and were required to maintain abstinence until blood sampling was completed. Study subjects were confined to the study facility from at least 36 hr prior to dosing until at least 24 hr after

	Nicotine 2 mg				Nicotine 4 mg			
	Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m^2)	Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m^2)
Number of observations	54	54	54	54	54	54	54	54
Mean	28	63.8	1.68	22.6	27	63.3	1.67	22.6
Standard Deviation	5	9.0	0.06	2.7	6	7.7	0.06	2.8
Median	28	62.8	1.68	22.5	26	62.1	1.67	22.0
Minimum	18	51.0	1.55	18.6	19	51.5	1.55	18.8
Maximum	43	83.3	1.83	27.2	41	83.2	1.81	29.6

Table 1: The demographics of all 54 recruited subjects in both the nicotine 2 mg and 4 mg studies are summarized.

dosing. Continued abstinence was monitored throughout the sample collection period with random carbon monoxide monitoring. Oral cavity examination and exhaled CO level were measured at before check-in (at least 36 hours prior to dosing) on Day-2; after check-in, at random times on Day -2 (at least 2 times) and Day-1 (at least 4 times); within 30 minutes prior to dosing (Day 1); at random times (at least 4 times) during the 24 hour blood sampling on Days 1 and 2 for each study period. All subjects had exhaled carbon monoxide levels less than 10 ppm in the morning prior to dosing. Each dosing period was separated by 8 days for 2 mg study and 9 days for 4 mg study, and subjects were permitted to smoke during this interval.

During the trial, the subjects were to remain ambulatory or seated upright for the first 4 hours after drug administration. During housing, post-dose meals were identical for both periods of the study. Lunch, snack and dinner were served at 4.0, 9.0 and 13.0 hours, respectively, after dosing. Water was not permitted from 1 hour before dosing until 1 hour following dosing, but it was allowed at all other times.

Adverse events were monitored throughout the study, until resolution or lost to follow-up. Adverse events were described in terms of severity, seriousness, outcome, action, frequency and relationship to treatments. The principal investigator or clinical-investigator was on-site, within the proximity of the subject confinement area after check-in and till check-out of all the subjects. Subjects were instructed to inform the study physician and/or nurses of any adverse events that occurred during the study.

Blood sampling

Blood samples (1 × 5 mL) for nicotine analysis were collected via an indwelling catheter (intra-venous) with respect to start time of chewing in vacutainers containing sodium heparin anticoagulant at -0.25, -0.17, -0.08 hours (pre-dose) and at 0.08, 0.17, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 2.00, 3.00, 6.00, 9.00, 12.00, 16.00 and 24.00 hours post dose [16]. After blood collection, vacuum collection tubes were inverted gently several times to ensure the mixing of tube content and blood sample. Tubes containing blood samples were immediately placed in an iced water bath at approximate temperature of 8-12°C till they were centrifuged. The blood sample tubes were centrifuged to separate plasma as soon as possible at 3000 rpm for 10 minutes in a centrifuge set at a temperature of 8°C. Then plasma was stored below -30°C at the clinical unit of Sitec Labs Pvt Ltd and then transferred to the bioanalytical facility of Sitec Labs Pvt Ltd under frozen condition and then samples were stored at -30°C or below until sample analysis.

To avoid contamination of blood samples with nicotine, study staff was not allowed to smoke in the study surroundings. Study staff was also asked to refrain from smoking in the morning before commencing work on the study. They were only permitted to be in contact with the samples after having washed their hands with soap and water. They were also double-gloved and were wearing nose masks, hair nets and lab coats.

Analytical methods

Plasma concentrations of Nicotine were assessed by a method using high-performance liquid chromatography with mass spectrometry detection (LC-MS/MS). An aliquot 500 µl of human plasma containing the analyte and the internal standard was extracted using a liquid - liquid extraction technique. The internal standard for Nicotine assay was Nicotine D3. 20 µl of the internal standard working solution was added to 500 µl of plasma sample. After vortexing the tubes, 50 µl of 10 M Potassium hydroxide solution was added and the tubes were again vortexed. To this tube 5 ml of Diethyl ether was added and vortexed for 3 min with pulsation. Samples were centrifuged for 3 min at 4000 rpm and then kept in freezer at -70°C for freezing the aqueous layer. Subsequently the organic layer was transferred to a tube containing 100 µl of 0.1% formic acid and vortexed for 3 mins. After vortexing, tubes were centrifuged at 4000 rpm and transferred to freezer at -70°C. After freezing the aqueous layer the samples were withdrawn from freezer and organic layer was removed. To the aqueous layer 700 µl of reconstitution solution was added. The reconstitution solution comprised of 10 µl of triethylamine in 100 mL of mobile phase. This final extract was transferred to glass vial for analysis using LC-MS/MS.

The extracts were injected into the LC-MS/MS system equipped with MDS Sciex API-4000 mass spectrometer. Positive ions were monitored in the multiple reaction-monitoring (MRM) mode. The following ion transitions using analyst 1.4.2 were monitored 163.2/130.4 and 166.2/130.1 for Nicotine and internal standard respectively. Linearity for Nicotine was assessed by plotting area ratios versus standard concentrations and using a linear regression weighted 1/concentration^2. Analytical range for Nicotine was 0.20-25 ng/mL. The column used for the analysis was Inertsil HILIC 15 cm × 4.6 mm, 3µ and the mobile phase composition was a mixture of acetonitrile, water and formic acid (90:10:0.75) and 10 mM Ammonium trifluroacetate. The retention time of Nicotine was 2.2 mins. Nicotine was chromatographically resolved from Anabasine which is a tobacco content and was detected in the same MRM ion channel as Nicotine. The blank plasma used for preparation of calibration standards and control samples was obtained from non-smoker subjects who were housed for three days and were provided control diet in order to reduce the Nicotine concentration in blood to acceptable level.

Method validation was performed according to the current international approach and the applicable regulations regarding bioanalytical method validation. The intra-batch and inter-batch accuracy and precision was evaluated at five different concentrations of control samples. The inter-batch accuracy ranged from 93.44 to 100.00% and the inter-batch precision ranged from 2.16 to 8.70%. The selectivity of the method was assessed by analyzing plasma samples from six normal and a haemolysed and lipemic source. Matrix effect was evaluated by performing post-extraction addition and post-column infusion experiments. Stabilities such as stock solution stability, short-term stability of analyte in plasma, freeze-thaw stability, post-preparative stability and long-term stability in plasma were assessed.

Pharmacokinetic analysis

The following PK parameters were calculated using validated PK software (WinNonlin version 6.3). The area under the curve from time zero to the last measurable concentration (AUC_{0-t}) using the linear trapezoidal rule, the area under the curve extrapolated to infinity (AUC_{0-t}+Clast /kel, where Clast is the last measurable plasma concentration), the maximum plasma concentration (C_{max}), and the time to maximum plasma concentration (t_{max}), the terminal rate constant of elimination (kel) and terminal elimination half-life ($t_{1/2}$). The ratio of AUC_{0-t}-$AUC_{0-\infty}$ ($AUC_{0-t}/AUC_{0-\infty}$) as well as the extrapolated area of the curve ($AUC_{0-\infty}$= ($AUC_{0-\infty}$- AUC_{0-t}) / $AUC_{0-\infty}$) were calculated as percentage.

Statistical analysis

A statistical analysis was performed using the SAS® GLM procedure (SAS® system for windows® release 9.2). Concentration values below the LOQ of the assay for nicotine (0.20 ng/mL) were set to zero. Analyses of variance (ANOVA) were performed on In-transformed AUC_{0-t}, and

C_{max} parameters. The ANOVA model included sequence, subjects nested within sequence, period and drug formulation as factors according to regulatory guidance on Bioequivalence. A statistical analysis was performed using the SAS® GLM procedure (SAS® system for windows® release 9.2) Geometric least-square means (LSM) as well as ratio of LSM with corresponding 90% confidence intervals (CI's) for the generic and innovator formulations were calculated. In addition, nonparametric methods were used to assess differences in median values of t_{max} between the two formulations and 90% CI's were constructed.

Results

Safety

A total of 54 subjects were recruited in nicotine 2 mg chewing gum study. There were 6 adverse events of mild and moderate severity. Overall, 4/54 (7.41%) subjects experienced an adverse event.

A total of 54 subjects were recruited in nicotine 4 mg chewing gum study. There were 21 adverse events of mild and moderate severity. Overall, 16/54 (29.63%) subjects experienced an adverse event.

No deaths or serious adverse events (SAE) occurred during conduct of both the studies. Adverse events of both the studies (2 mg and 4 mg) are summarized in Table 2.

During vital signs examination, there were no clinically significant deviations observed from the baseline values and no clinically significant changes were noted in post-study clinical laboratory data. All subjects were found fit in post-study examination. There were no clinically significant changes observed in post-study ECGs when compared with pre-study ECGs.

Pharmacokinetics and statistics

A total of 54 subjects were recruited in nicotine 2 mg chewing gum study, but only 47 subjects completed the study. 2 subjects dropped out from the study before dosing of period-1 and 5 subjects dropped out from the study before dosing of period-2 for personal reasons. The plasma samples of 52 subjects were analyzed for nicotine except the subjects who were dropped out from the study before dosing of period-1. Pre-dose concentration levels of nicotine of 4 subjects were greater than 5% of the C_{max} in Period-2. Therefore, data of 4 subjects was not considered for final pharmacokinetic and statistical analysis. Data of remaining 43 subjects was considered for pharmacokinetic and statistical analysis. For Nicotine concentrations which were quantifiable at pre-dose sampling (which were <5% of C_{max}), all parameters were calculated after correction for individual pre-dose levels in the secondary analysis. Bioequivalence acceptance criteria concluded was based on without baseline-adjusted results only.

A total of 54 subjects were recruited in nicotine 4 mg chewing gum study, but only 40 subjects completed the study. 11 subjects dropped out from the study before dosing of period-2 for personal reasons. 3 subjects were discontinued from the study due to an adverse event. The plasma samples of all the 54 subjects were analyzed for nicotine. Pre-dose concentration levels of nicotine of 3 subjects were greater than 5% of the C_{max} in Period-2. Therefore, data of 3 subjects was not considered for final pharmacokinetic and statistical analysis. Data of remaining 37 subjects was considered for pharmacokinetic and statistical analysis. For Nicotine concentrations which were quantifiable at pre-dose sampling (which were <5% of C_{max}), all parameters were calculated after correction for individual pre-dose levels in the secondary analysis. Bioequivalence acceptance criteria concluded was based on without baseline-adjusted results only.

The blood samples were collected up to 24 hrs. post dose. Mean plasma concentration profiles of nicotine (without baseline adjusted) under linear over the 24-hour pharmacokinetic study are presented in Figure 1 (for 2 mg study) and Figure 2 (for 4 mg study). Overall, mean plasma concentrations of nicotine peaked rapidly and then declined in a mono-exponential manner, with some plasma concentration values falling not below the LOQ of the assay at 24 hours post dose. Therefore, 1-2 additional time points were required after 24 hours post dose. Values below the LOQ were set to zero for pharmacokinetic analysis. A 36 hour period of abstinence from smoking prior to dosing was not sufficient since pre dose concentration levels of nicotine of 4 subjects (for 2 mg study) and 3 subjects (for 4 mg study) were greater than 5 percent of the C_{max} in Period 2. Mean plasma concentrations of nicotine following oral administration of these formulations were almost superimposable during the early absorption, distribution and elimination phases of the products. Ratios of $AUC_{0-t}/AUC_{0-\infty}$ for all subjects were found to be more than 80%, indicating that blood samples collected adequately characterized the pharmacokinetic profile of the drug. In addition, 43 subjects provided >90% power to detect a difference of at least 20% in C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ between the two treatments for 2 mg study; and 37 subjects provided >99% power to detect a difference of at least 20% in C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ between the two treatments for 4 mg study.

The statistical results of the primary pharmacokinetic parameters of nicotine (2 mg and 4 mg) (without baseline adjusted) are presented in Table 3. The geometric mean ratios, 90% CI, power and intra subject coefficient of variation of test and references for Ln transformed

Adverse Event (Preferred Term)	Frequency (Percentage)	Relationship	Number of Adverse Events	
			Test product (T)	Reference product (R)
Nicotine 2 mg				
Dizziness	7.41%	Related	2	2
Pyrexia	1.85%	Not Related	0	1
Pruritus	1.85%	Not Related	0	1
Nicotine 4 mg				
Dizziness	16.67%	Related	4	5
Nausea	9.26%	Related	1	4
Headache	5.56%	Related	1	2
Ear pain	1.85%	Not Related	0	1
Nasopharyngitis	1.85%	Not Related	0	1
Eye pain	1.85%	Not Related	1	0
Vomiting	1.85%	Related	1	0

Table 2: Adverse events of both the nicotine 2 mg and 4 mg studies are summarized.

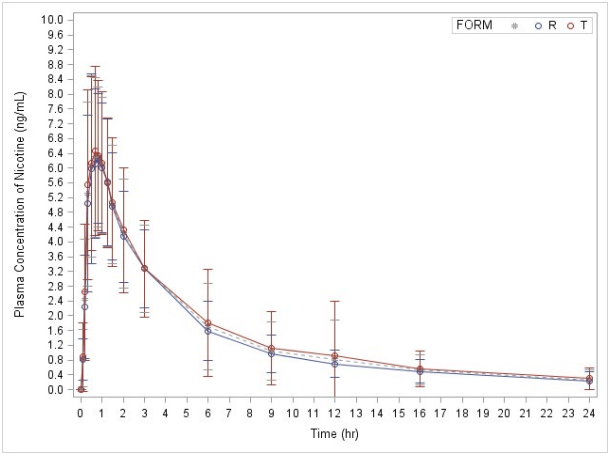

Figure 1: Mean graph (linear) for plasma concentration vs. time profile of nicotine after oral dose of nicotine 2 mg chewing gum (without baseline adjusted). Each point represents the mean+standard deviation. (n=43). O: (Reference) NICORETTE® Freshmint 2 mg Gum; O: (Test) Nicotine 2 mg Mint Flavored Chewing Gum.

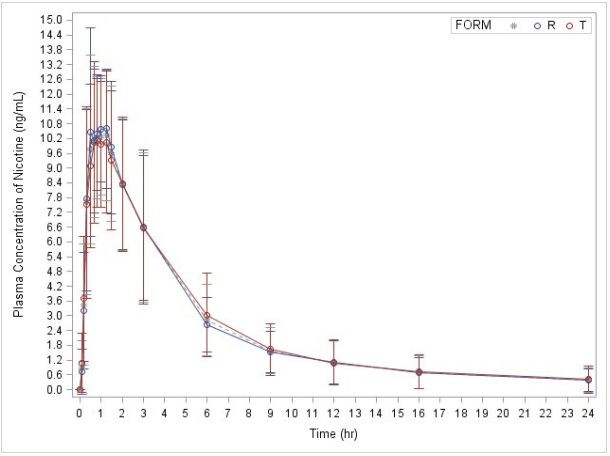

Figure 2: Mean graph (linear) for plasma concentration vs. time profile of nicotine after oral dose of nicotine 4 mg chewing gum (without baseline adjusted). Each point represents the mean+standard deviation. (n=37). O: (Reference) NICORETTE® Freshmint 4 mg Gum; O: (Test) Nicotine 4 mg Mint Flavored Chewing Gum.

Pharmacokinetic Parameters	2 mg		4 mg	
	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)
N	43	43	37	37
C_{max} (ng/ml)	7.68 ± 2.33	7.26 ± 2.07	12.43 ± 3.37	13.09 ± 3.65
AUC_{0-t} (hr.ng /ml)	35.34 ± 19.93	32.30 ± 11.49	56.61 ± 26.16	55.80 ± 20.31
$AUC_{0-\infty}$ (hr.ng /ml)	41.13 ± 22.35	36.63 ± 13.44	65.41 ± 44.45	62.72 ± 31.72
Ratio of $AUC_{0-t}/AUC_{0-\infty}$	0.87 ± 0.10	0.89 ± 0.06	0.91 ± 0.09	0.92 ± 0.08
*T_{max} (hr)	0.67 (0.33-1.50)	0.67 (0.33-3.00)	1.00 (0.33-3.00)	0.83 (0.33-3.00)
K_{el} (1/hr)	0.122 ± 0.080	0.131 ± 0.077	0.145 ± 0.079	0.151 ± 0.084
$T_{1/2}$ (hr)	8.65 ± 6.42	7.44 ± 4.71	7.38 ± 6.75	6.80 ± 5.49

*Median (range)

Table 3: The statistical results of primary pharmacokinetic parameters of nicotine (2 mg and 4 mg) (without baseline adjusted) are presented.

pharmacokinetic parameters C_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$ for nicotine (2 mg and 4 mg) are presented in Table 4.

Nicotine in chewed cuds were analyzed for residual nicotine content. Based on gum cud analysis, the average release of nicotine was similar for the two investigational products (78% for the test product and 69% for the reference product).

Discussion

In these studies, we investigated the bioequivalence of two test and two reference formulations of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gum. From a consumer perspective, chewing gum is a discrete dosage form that tastes good, is widely accepted and consumed routinely in its confectionery form. Mint flavored chewing gums were selected because of better taste. From a technical perspective, local delivery in the mouth enables fast uptake of nicotine by buccal absorption.

Assessment of bioequivalence of generic product to reference product is required to exclude any clinically important differences in the rate or extent at which the active entity of the drugs becomes available at the site of action. Two medicinal products containing the same active substance are considered bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and their bio-availabilities (rate and extent) after administration in the same molar dose lie within acceptable predefined limits. These limits are set to ensure comparable *in vivo* performance, i.e., similarity in terms of safety and efficacy [17].

Both the studies demonstrate generic and innovator formulations of both nicotine 2 mg and 4 mg chewing gum displayed similar rate and extent of bioavailability of nicotine. The median T_{max} for both test and reference was found to be 0.67 hr. for 2 mg study. The T_{max} is comparable.

The median T_{max} for test and reference was found to be 1.00 hr and 0.83 hr respectively for 4 mg study. Wilcoxon-Mann-Whitney two sample tests for difference in median T_{max} were performed using SAS® 9.2. The difference between the median T_{max} of Test and Reference product is not statistically significant.

The C_{max} was found to be consistent both for test and reference in both the studies, indicating the attainment of similar body peak levels. The mean data are also comparable. For the AUC parameter, the results were found to be similar and there was not much difference in inter-subject variability. The $T_{1/2}$ values are also comparable and in the elimination phase there is no variation.

The statistical analysis was carried out for both untransformed and log transformed data. The data showed statistical equivalence for the important pharmacokinetic parameters i.e. C_{max}, and AUC_{0-t}. A power of >90% was achieved for the pharmacokinetic parameters for both 2 mg and 4 mg studies.

Considering that all 90% CIs of the ratios of the pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were found to be within the predetermined ranges of bioequivalence and that the two one-sided t tests found all of the probability values to be <0.05, the results of

Pharmacokinetic Parameters	Geometric Mean		*(%)T/R	90% Confidence Interval	Power (%)	Intra subject CV%
	Test	Ref				
Nicotine 2 mg						
N	43	43	-	-		
C_{max} (ng/ml)	7.2018	6.9848	103.11	93.71-113.45	98.49	26.77
AUC_{0-t} (hr.ng/ml)	30.4278	30.2235	100.68	89.95-112.68	94.69	31.76
$AUC_{0-\infty}$ (hr.ng/ml)	35.4458	34.1476	103.80	92.90-115.98	95.18	31.28
Nicotine 4 mg						
N	37	37	-	-	-	-
C_{max} (ng/ml)	12.0187	12.5898	95.46	88.68 - 102.76	99.92	18.90
AUC_{0-t} (hr.ng/ml)	51.9601	52.0334	99.86	91.48 -109.00	99.34	22.55
$AUC_{0-\infty}$ (hr.ng/ml)	57.0800	56.5841	100.88	91.14-111.66	97.46	26.26

*(%) T/R is ratio of Test Geometric Mean/Ref Geometric Mean

Table 4: The Geometric mean ratios, 90% CIs, power and intra subject coefficient of variation of test and reference for Ln transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for nicotine (2 mg and 4 mg) (without baseline adjusted) are presented.

both studies satisfied the accepted regulatory requirements to assume bioequivalence.

The intra-subject CV was found to be 26.77 % for C_{max}, 31.76 % for AUC_{0-t} and 31.28 % for $AUC_{0-\infty}$ for log-transformed data for 2 mg study.

The intra-subject CV was found to be 18.90 % for C_{max}, 22.55 % for AUC_{0-t} and 26.26 % for $AUC_{0-\infty}$ for log-transformed data for 4 mg study.

The sample size of 54 subjects selected for both the studies was considered to be sufficient to provide adequate power to meet bioequivalence criteria. To avoid variability in the study, to minimize adverse events and to increase compliance healthy adult male human smoker subjects were selected. All the subjects were dosed between 08:00 to 09:46 in both the periods.

During the clinical study there were no significant protocol/standard operating procedure (SOP) deviations and adverse events were mild to moderate in nature. The subjects tolerated the study medications well. During the study subject compliance to restriction of use of tobacco products was checked by oral cavity examination and exhaled CO level measurements, and all subjects were found compliant. During oral cavity examination no illicit use of tobacco products was found, and exhaled CO level measurements after check-in were <10 ppm for all the subjects. The biological samples were successfully analyzed by LCMS/MS. The quality control data are found to be consistent and precise.

Conclusion

The 90% CI of Nicotine for C_{max} and AUC_{0-t} were within 80.00-125.00% for both the studies, suggesting the two generic formulations of Nicotine 2 mg and 4 mg Mint Flavored Chewing Gum were bioequivalent with the two innovator formulations of NICORETTE® Freshmint 2 mg and 4 mg nicotine, as nicotine resinate, mint flavored chewing gum of MCNEIL CONSUMER HEALTHCARE GMBH.

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Conflict of Interest: All authors are employees of Sitec Labs. Pvt. Ltd. The authors have indicated that they have no other conflicts of interest regarding the content of the article.

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