

A Bioequivalence Study of Nicotine 2 Mg Lozenges in Indian Healthy Adult Human Male Smoker Subjects

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Abstract

Nicotine Lozenges are used to aid smokers wishing to quit or reduce prior to quitting. The aim of this study was to determine the bioequivalence of a test and reference formulation of Nicotine 2 mg Lozenge. This single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design study was conducted in 28 healthy adult Indian male subjects under fasting conditions with a washout period of 7 days. Study formulations were administered after a 10-hour overnight fast. Blood samples for pharmacokinetic profiling were taken post-dose up to 16 hours. Safety was evaluated through the assessment of adverse events, and laboratory tests. Plasma concentration of Nicotine was 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 AUC_{0-t} and C_{max} values for the test and reference products, using logarithmic transformed data. The 90% confidence intervals of Nicotine were 96.16-119.10 and 92.16-111.51 respectively. Since the 90% confidence intervals for C_{max} and AUC_{0-t} were within the 80 – 125% interval, it was concluded that the two formulations of Nicotine 2 mg Lozenge are bioequivalent in their rate and extent of absorption.

Keywords: Nicotine 2 mg lozenge; Smoking cessation; Nicotine replacement therapy; Bioequivalence

Introduction

Smoking accounts for more deaths and diseases worldwide than any other modifiable risk factors [1,2]. Literature suggests that approximately three quarters of smokers want to quit; however, smoking is highly addictive and smoking cessation is difficult with frequent relapses common amongst those who try to quit [1]. There is ongoing research on the effectiveness of various smoking cessation interventions. Nicotine replacement therapy (NRT), bupropion, varenicline and cytisine medications have been shown to improve the likelihood of quitting, with varenicline showing the greatest benefit. However, these products have relatively low consumer appeal and product satisfaction [3,4]. Evidence suggests that psychosocial smoking cessation interventions such as behavioural counselling, telephone support and self-help interventions are effective; behavioral interventions combined with pharmacotherapy increase the success rate of quitting [5,6].

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 [7]. Nicotine replacement therapy is available in several forms- patches, nasal sprays, inhalers, sublingual tablets, lozenges, chewing gums and electronic cigarettes. Nicotine lozenges are the clinician's preferred form of nicotine replacement for users of chewing tobacco and snuff.

A new generic formulation of Nicotine 2 mg Lozenge has been developed having the same composition as innovator brand, NIQUITIN[®] 2 mg Lozenge of GLAXOSMITHKLINE CONSUMER HEALTHCARE, UK. A single dose of Nicotine 2 mg Lozenge has been evaluated in this study. The pharmacokinetics of Nicotine were evaluated in 28 healthy male volunteers. The aim of this study was to determine the Bioequivalence and to compare the pharmacokinetics of two formulations (innovator vs. generic) of Nicotine 2 mg Lozenge.

Materials and Methods

Study design

This study was an open label, randomized, two-treatment, two sequence, two-period, cross-over, single-dose comparative oral bioavailability study of Nicotine 2 mg Lozenge (Test) and NIQUITIN[®] 2 mg Lozenge (Reference) of GLAXOSMITHKLINE CONSUMER HEALTHCARE, UK.

All study medications were kept in a pharmacy and temperature and humidity were monitored continuously. A SAS generated randomization code was used to ensure balanced permutation of the treatments.

All volunteers were adult male smokers who were in generally good health. 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 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 (Day 1), 6 pre-dose readings as per randomization (Day 2), on dosing day prior to dosing (within 30 minutes) (Day 3) and 4 post-dose readings as per randomization (Day 3) for each study period. All subjects had exhaled carbon monoxide levels less than 10

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ppm in the morning prior to dosing. Each dosing period was separated by 7 days, and subjects were permitted to smoke during this interval. Training sessions on the appropriate technique of dose administration were conducted prior to each dosing period. Drug administration was standardized as follows: lozenges were moved from side to side in the mouth every 4 seconds until completely dissolved. The movement of the lozenge were marked by a timer with an audible signal, and swallowing was timed with a verbal command given every 30 seconds. The dissolution times for the lozenge were recorded.

During the trial, the subjects were to remain ambulatory or seated upright for the first 2 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.

The protocol and informed consent forms (ICFs) were reviewed and approved prior to study initiation by an independent ethics committee. All subjects read and signed the ICF prior to study initiation. This clinical trial was conducted in accordance with the Declaration of Helsinki, good Clinical Practice guidelines and national regulatory requirements [8-10].

Subjects and treatments

The subjects were screened within 21 days prior to study enrolment. The screening procedure included medical histories and demographic data, including name, sex, age, race, body weight (kg), height (cm), and tobacco use. Healthy adult male volunteers (light smokers, who smoke ≤ 10 cigarettes per day regularly since last three months) were to fulfill all of the following inclusion criteria to be eligible for participation in the study. Males with a minimum age of 18 years and body mass index greater than or equal to 18.5 kg/m² and less than or equal to 30.00 kg/m². All subjects were subjected to a vital signs measurement, a 12-lead electrocardiogram (ECG), and laboratory tests to evaluate their hematologic, hepatic and renal functions, prior to study enrolment, the clinical investigator reviewed the screening data and performed the physical examinations. The subjects were not to consume any food and beverages containing xanthines or alcohol (48 hours before dosing and throughout the period of sample collection), grapefruit (7 days before dosing and throughout the study), or vitamins (throughout the confinement period). Medication (including herbal and over-the-counter products) was 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. A total of 28 healthy adult male volunteers who had satisfied the above screening criteria were admitted to the study center in the evening before dosing (Day-1), and then they were assigned to each treatment sequence as per the randomization scheme. All the subjects received doses of Nicotine 2 mg Lozenge on the dosing day. Adverse events were monitored throughout the study, until resolution or loss to follow-up. Adverse events were described in terms of severity, seriousness, outcome, action, frequency and relationship to treatments. The principal investigator or sub-investigator was on-site, within the proximity of the subject confinement area for first 6 hours after drug administration. Subjects were instructed to inform the study physician and/or nurses of any adverse events that occurred during the study.

Blood samples (1 x 5 ml) for nicotine analysis were collected in

vacutainers containing sodium heparin anticoagulant at hour 0.00 (pre-dose) within 15 minutes prior to dosing and at 0.08, 0.17, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 2.00, 3.00, 4.00, 6.00, 9.00, 12.00 and 16.00 hours post dose. Immediately, blood samples were centrifuged under refrigeration and then plasma was separated and 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, all the staff were not allowed smoking in the study surroundings. Staff working on the study were 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 μ l 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 μ l of the internal standard working solution was added to 500 μ l of plasma sample. After vortexing the tubes, 50 μ l 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 μ l 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 μ l of reconstitution solution was added. The reconstitution solution comprised of 10 μ l 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 160.2/130.1 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/concentration². Analytical range for Nicotine was 0.20 - 25 ng/ml. The column used for the analysis is Inertsil HILIC 15 cm x 4.6 mm, 3 μ and the mobile phase composition was a mixture of acetonitrile, water and formic acid (90:10:0.75) and 10 mM Ammonium trifluoroacetate. The retention time of Nicotine is 2.2 mins. Nicotine was chromatographically resolved from Anabasine which is a tobacco content and is 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.

Pharmacokinetics and statistical analysis

The following PK parameters were calculated using validated PK software (WinNonlin version 5.3). 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 ($AUC_{0-\infty} + C_{last}/k_{el}$, where C_{last} 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 (k_{el}) 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.

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 (CIs) 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% CIs were constructed.

Results

Demographics and safety results

28 male smoker subjects representing the general population were enrolled in this study, but only 27 subjects completed the study. The demographics of all 28 recruited subjects are summarized in Table 1.

No deaths or serious adverse events occurred during conduct of this study. There were 21 adverse events of mild and moderate severity. Overall, 11/28 (39.29%) subjects experienced an adverse event. One subject was discontinued due to adverse event (nausea, vomiting and giddiness). Adverse events are summarized in Table 2.

Pharmacokinetics and statistics

Mean plasma concentration profiles of nicotine under linear over the 16-hour pharmacokinetic study are presented in Figure 1. Overall, mean plasma concentrations of nicotine peaked rapidly and then declined in a monoexponential manner, with some plasma concentration values falling not below the LOQ of the assay at 16 hours post dose. Therefore, one more time point was required after 16 hours

	Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m ²)
Number of observations	28	28	28	28
Mean	25	65.6	1.69	23.0
Standard Deviation	6	8.4	0.06	2.5
Median	23	65.1	1.68	23.0
Minimum	18	54.7	1.57	18.8
Maximum	40	89.0	1.80	28.1

Table 1: The demographics of all 28 recruited subjects are summarized.

Adverse event	Frequency (percentage)	Relationship	Number of adverse events	
			Test product (T)	Reference product (R)
Giddiness	25%	Related	7	0
Nausea	17.86%	Related	2	3
Headache	14.29%	Related	3	1
Abdominal pain	7.14%	Related	0	2
Vomiting	3.57%	Related	1	0
Neck pain	3.57%	Not Related	0	1
Pain at cannulation site	3.57%	Not Related	1	0

Table 2: Adverse events are summarized.

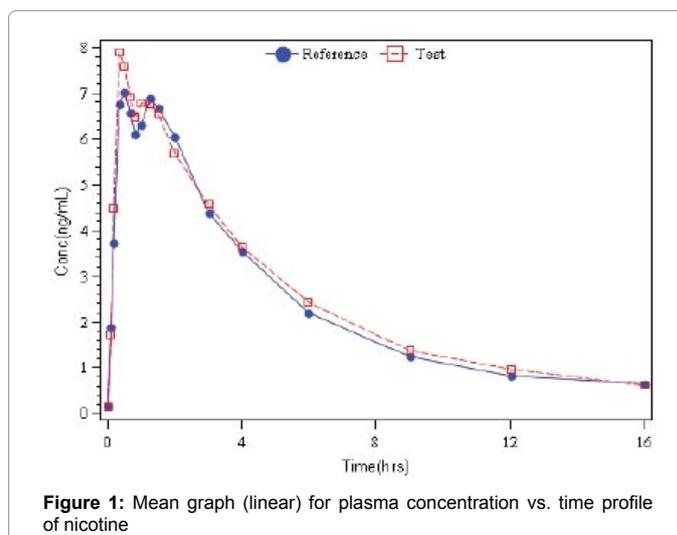


Figure 1: Mean graph (linear) for plasma concentration vs. time profile of nicotine

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 6 subjects were greater than 5 percent of the C_{max} in Period 2. Therefore, 6 subjects were not considered for final pharmacokinetic and statistical evaluation.

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 the subjects found to be more than 80% indicating the blood samples collected adequately characterized the pharmacokinetic profile of the drug. In addition, 21 subjects provided >90% power to detect a difference of at least 20% in C_{max} and AUC_{0-t} between the two treatments.

The statistical results of primary pharmacokinetic parameters of nicotine are presented in Table 3. The Geometric mean ratios, 90% CIs, power and intra subject coefficient of variation of test and references for Ln transformed pharmacokinetic parameters C_{max} and AUC_{0-t} for nicotine are presented in Table 4. The 90% CIs for nicotine were within 80.0-125.0%, suggesting the generic of Nicotine 2 mg Lozenge is bioequivalent with NiQuitin' 2 mg Lozenge of GlaxoSmithKline Consumer Healthcare, UK.

Discussion and Conclusion

The current investigation demonstrates both generic and innovator formulations displayed similar rate and extent of bioavailability of nicotine. The median T_{max} for test and reference is found to be 0.50 hr.

Pharmacokinetic Parameters	Mean ± SD	
	Test (T)	Reference (R)
N	21	21
C _{max} (ng/ml)	9.18 ± 2.87	8.52 ± 2.43
AUC _{0-t} (hr.ng /ml)	39.59 ± 19.78	37.54 ± 12.27
AUC _{0-∞} (hr.ng /ml)	45.14 ± 26.60	42.46 ± 14.93
*T _{max} (hr)	0.50 (0.33-1.50)	0.50 (0.33-2.02)
K _{el} (1/hr)	0.179 ± 0.069	0.191 ± 0.060
T _{1/2} (hr)	4.54 ± 2.05	3.97 ± 1.22

*Median (range)

Table 3: The statistical results of primary pharmacokinetic parameters of nicotine are presented

Pharmacokinetic Parameters	Geometric Mean		*(%) T/R	90% Confidence Interval	Power (%)	Intra subject CV%
	Test	Ref				
N	21	21	-	-		
C _{max} (ng/ml)	8.7850	8.2091	107.01	96.16-119.10	96.20	20.23
AUC _{0-t} (hr. ng/ml)	36.1669	35.6764	101.37	92.16-111.51	98.42	17.97

*(%) 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 references for Ln transformed pharmacokinetic parameters C_{max} and AUC_{0-t} for nicotine are presented

The T_{max} is comparable. The C_{max} is found to be consistent both for test and reference indicating the attainment of body peak levels similarly. However the mean data is very much comparable. For the AUC_{0-t} parameter the results found to be similar and 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}. The 90% confidence intervals are well within the limits and can be acceptable by any regulatory agency. A power of >90% was achieved for the pharmacokinetic parameters. The intra subject CV was found to be 20.23 % for C_{max}, and 17.97 % for AUC_{0-t} for log transformed data. Based on the results the generic is found to be bioequivalent and can be substituted for brand product.

The present investigation has been successfully conducted in 28 healthy male volunteers. During the clinical study there were no significant protocol/SOP deviations and adverse events were mild in nature. The subjects tolerated the study medication well. The biological samples were successfully analysed by LCMS/ MS. The quality control data is found to be consistent and precise.

As a result, the generic formulation of nicotine 2 mg Lozenge should be equally effective and safe as the innovator product of NiQuitin® 2 mg Lozenge of GlaxoSmithKline Consumer Healthcare, UK.

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