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ORIGINAL INVESTIGATION

Characterization of the nicotine uptake and safety of Nordic spirit tobacco-free oral nicotine pouches: A randomized cross-over study

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Abstract

Rationale Given the nascency of tobacco-free oral nicotine pouches (NPs) and the heterogeneity of commercially available NP brands, there is a need for scientific evaluation of different NP formulations. Nordic Spirit, novel NPs are distinguished by their unique composition.

Objectives To characterize blood nicotine delivery, pharmacokinetics (PK), subjective and physiological effects and to monitor safety of three Nordic Spirit NPs (6 mg, 9 mg and 11.2 mg/pouch) compared with LD tobacco snus (11.2 mg/pouch) and Nicorette® gum (4 mg/unit) following single 30 min use.

Methods This was a randomized, open-label, ten-sequence, single-use, cross-over clinical study with 30 healthy adult Swedish snus users.

Results Peak nicotine concentrations (C_{max}) ranged from 10.92 to 17.32 ng/mL for the three Nordic Spirit NPs, with a trend toward dose proportionality, and 8.18 ng/mL and 9.23 ng/mL for the LD snus and Nicorette® gum comparators, respectively. Peak concentration for Nordic Spirit NPs was reached (T_{max}) after 30 to 38 min, and after 45 min for LD snus and Nicorette® gum. No notable safety concerns were observed after single use for any of the study products.

Conclusions Delivery of nicotine from the three Nordic Spirit NPs appeared to be nicotine content-dependent, based on C_{max} and AUC. The amount of nicotine extracted showed positive correlation with the reported C_{max} and AUC. For Nordic Spirit NPs, T_{max} was immediately after end of use. The characteristics of Nordic Spirit NPs were found to be favourable for profiling NP nicotine delivery and safety in human use, and for further product development.
ISRCTN registry study no. ISRCTN75583947.

Keywords Nicotine pouches · Nicotine · Nicotine pharmacokinetics · Oral nicotine delivery · Subjective measures · Tobacco-free nicotine pouches · Safety

Abbreviations

AE	Adverse event
ANOVA	Analysis of variance
AUC	Area of the plasma concentration *vs.* time curve
BMI	Body mass index
CI	Confidence interval
ECG	Electrocardiogram
EU	European Union
ISRCTN	International standard randomized controlled trial number
JT	Japan Tobacco
JTI	JT International SA
LLOQ	Lower limit of quantification
LS	Least squares
NP	Nicotine pouch
NRT	Nicotine replacement therapy
PES	Product evaluation scale
PK	Pharmacokinetics
SAE	Serious adverse event
VAS	Visual analog scale

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Introduction

Cigarette smoking is a cause of smoking-related diseases such as lung cancer, coronary heart disease, emphysema, and chronic bronchitis (United States Department of Health and Human Services 2014). Although not risk free, nicotine is not the primary cause of smoking-related diseases (Gottlieb and Zeller 2017). The highest risk of disease comes from the long-term exposure to the high levels of harmful constituents produced by tobacco leaf combustion and inhalation of the smoke (Institute of Medicine 2001; Dai et al. 2022). By eliminating tobacco combustion and the generation of smoke, non-combustible tobacco- and nicotine-containing products, have the potential to be substantially less harmful compared with cigarettes as they contain or produce fewer and substantially lower levels of harmful chemicals.

Tobacco-free oral nicotine pouches (NPs) have recently been launched in various markets globally. NPs are small pouches that are often composed of nicotine, non-tobacco filler materials, and food-grade quality additives. NPs have a similar appearance and usage to pre-portioned Swedish snus, where nicotine is absorbed through the oral mucosa by placing the product between the gum and lip. However, unlike snus, NPs do not contain tobacco leaf (Azzopardi et al. 2022b). Given NPs do not contain or burn tobacco, recent scientific evidence indicates a reduced risk potential for this product category (Patwardhan and Fagerström 2022). Chemical characterization studies have shown NPs contain toxicant levels comparable to levels in nicotine replacement therapies (NRTs) (Azzopardi et al. 2022b; Back et al. 2023) which in turn leads to substantially reduced in vitro toxicological activity compared with cigarette smoke (Miller-Holt et al. 2022; Yu et al. 2022). Moreover, recent clinical data has shown the use of NPs is associated with a substantial reduction in exposure to harmful chemicals compared to cigarette smoking with reductions comparable to never smoker controls (Azzopardi et al. 2023; Rensch et al. 2023).

To date, a limited number of studies have been published in the scientific literature assessing the nicotine PK, subjective effects and safety associated with NPs use. Two clinical studies assessed NP blood nicotine delivery compared to smokeless tobacco products (Lunell and Curvall 2011; Liu et al. 2022), while other studies have assessed NP use with conventional cigarettes (Rensch et al. 2021; McEwan et al. 2022; Liu et al. 2022; Chapman et al. 2022) or NRT products (Azzopardi et al. 2022a) as comparators. Lunell et al. 2020 concluded that two marketed NPs (containing 6 and 8 mg nicotine) delivered nicotine as quickly and to a similar extent as existing smokeless products, with no significant adverse effects. Liu et al. 2022 reported that nicotine delivery and subjective effects of the NPs they assessed were not likely to be higher than for combustible cigarettes or smokeless tobacco products.

Given the nascency of the NP category, and the heterogeneity of commercially available NPs, there is a need for further scientific evaluation of different NP formulations to characterize nicotine delivery, subjective effects, and safety. Nicotine sources, non-tobacco fillers, pouch size, materials, and additives vary amongst commercial NP brands. As a result, physicochemical properties such as powder particle size, pH, and moisture differ depending on the formulation which may impact nicotine extraction and buccal absorption into the blood stream. Beyond the relationship between nicotine content and nicotine release (Aldeek et al. 2021; Stanfill et al. 2021; McEwan et al. 2022), the impact of other physicochemical properties on blood nicotine delivery from oral nicotine products remains incompletely understood (Pickworth et al. 2014). Therefore, the nicotine PK profile of one NP brand may not be generalizable to other NPs with different formulations and different physicochemical properties and so further scientific data is required to close this knowledge gap.

To this end, the present study aimed to characterize the blood nicotine delivery, pharmacokinetics (PK), subjective and physiological effects, and safety of commercially available Nordic Spirit NPs at three different nicotine contents (6 mg, 9 mg and 11.2 mg per 0.65 g pouch). Nordic Spirit NPs contain plant-based fibres and a gum base filler, food-grade quality additives, and a pharmaceutical-grade nicotine polymer (nicotine Polacrilex 20%). While Nordic Spirit NPs share similar properties with other previously reported NP brand assessments, they are distinguished by their unique formulation that includes a complex of nicotine and Polacrilex polymer and use of a gum base as the filler material. Here we conducted a randomized, open-label, 5-period, ten-sequence, cross-over clinical study in 30 healthy adult Swedish snus users following a single 30 min period of use of Nordic Spirit NPs. A traditional Swedish pre-portioned snus (LD; 11.2 mg per pouch) was included as a comparator, considering its characteristics having a long history in markets, delivering nicotine orally and containing similar amount of nicotine as Nordic Spirit NPs. A 4 mg Nicorette® gum was also included as a comparator, since the product was qualitatively close to Nordic Spirit NPs.

Materials and methods

Study design

An overview of study design is provided in Fig. 1.

This was an open-label, randomized, 5-period, ten-sequence, single use, cross-over study conducted at a single

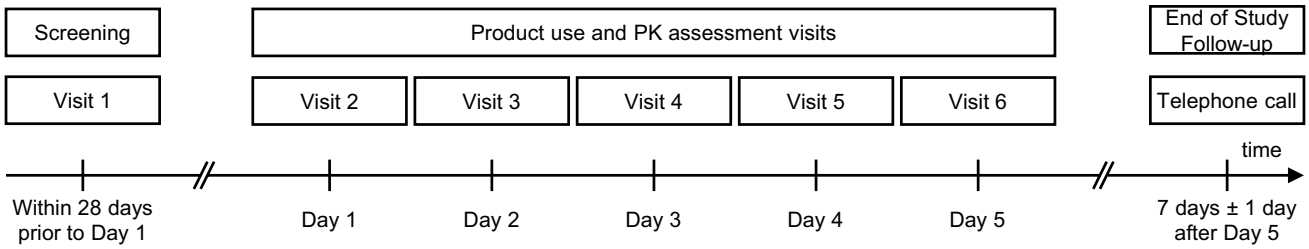

Fig. 1 Study design overview

site (Clinical Trial Consultants AB, Uppsala, Sweden). The study included a screening visit, five product use and assessment visits, and a follow-up phone call 6–8 days after the last study product use. Product use visits were separated by washout periods of at least 24 h between the start of each product use to avoid carryover effects. Screening occurred within 28 days prior to the first product use and consisted of an eligibility assessment, review of health status and nicotine/tobacco use history. Baseline characteristics of the participants, such as gender, age, body mass index (BMI), their daily consumption of snus and nicotine content of their usual brand of snus, were collected at screening.

Participants were randomly assigned to one of ten sequences, balanced with three participants per sequence, according to a computer-generated randomization list, prior to the first product use. The original randomization list was kept by the randomizer. The use of any nicotine-containing products aside from the study products was not allowed from 12 h before the start of each product use and until the participants left the study site. Additionally, participants were not allowed to change their usual brand of snus during the study. At each product use and assessment visit, after 30 min of use (see Study products below), the used Nordic Spirit and LD snus pouches were collected and frozen at −20 °C within 5 min for later measurement of residual nicotine. Participants were instructed not to eat, drink, chew gum or brush their teeth from 30 min before product use, during product use and until 30 min after the end of product use. Blood samples for PK analysis were collected at pre-determined time points from pre-use up to 8 h after the start of product use. Vital signs, 12-lead electrocardiograms (ECG) and self-assessment of subjective product experience were also recorded at pre-determined time points. End of product use safety assessments (see Study assessments below) were performed after the last PK blood sampling during the last visit to the clinical site.

Participants

Healthy male and female Swedish portion snus users aged 19–64 years with a BMI ≥ 18.5 and ≤ 30.0 kg/m^2 were eligible for study participation. Participants were exclusive and daily (7 days per week) users of Swedish snus with nicotine contents ≥ 8 mg/pouch for > 6 months prior to screening (single, occasional smoking of conventional cigarettes within 14 days prior to screening was allowed) and had urine cotinine levels > 200 ng/mL at screening. Prospective participants who planned to quit nicotine/tobacco use during the study period, or who would postpone an attempt to quit to participate in the study were excluded. Full details of eligibility criteria are available from https://doi.org/10.1186/ISRCTN75583947. Study participants were informed that they were free to quit nicotine/tobacco use and withdraw from the study at any time, for any reason, without forfeiting the right to appropriate follow-up. In total, 67 prospective participants were screened, and 30 were enrolled in the study.

Study products

All study products were commercially available in Sweden at the time of the study. The products evaluated were: (a) Nordic Spirit mint-flavored NPs containing 6 mg nicotine/pouch; (b) Nordic Spirit mint-flavored NPs containing 9 mg nicotine/pouch; (c) Nordic Spirit spearmint-flavored NPs containing 11.2 mg nicotine/pouch; (d) conventional Swedish pre-portioned snus, bergamot-flavored LD Original Vit Stark portion containing 11.2 mg nicotine/portion; and (e) an NRT mint-flavored nicotine gum, Nicorette®, containing 4 mg nicotine/unit. Nordic Spirit NPs and LD snus were manufactured by Nordic Snus AB (a JT International SA company) and Nicorette® gum was manufactured by McNeil AB.

Study participants used each of the five products, in a randomized order, on separate days. the participants were instructed to place the Nordic Spirit NPs and LD snus between the upper lip and gum and to keep it in position for 30 min. For the Nicorette® gum, participants were instructed to chew the gum slowly ad libitum for 30 min.

Study assessments

Pharmacokinetics (PK) analyses

Venous blood samples were collected within 10 min prior to each study product use as well as at the following time

points after the start of product use: 5, 8, 10, 15, 20, 30, and 45 min, as well as 1, 1.5, 2, 3, 4, 6, and 8 h. Separated plasma samples were analyzed for nicotine concentrations by Lablytica Life Science AB (Uppsala, Sweden) by means of a validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) method. This method was validated according to "Guideline on bioanalytical method validation, European Medicines Agency, 2011 (21 July 2011, EMEA/CHMP/EWP/192217/2009, Committee for Medicinal Products for Human Use (CHMP). PK parameters were calculated using non-compartmental analysis using Phoenix WinNonlin® version 8.1 (Certara, Princeton NJ, United States). The maximum observed nicotine concentration in plasma (C_{max}) and time to C_{max} (T_{max}) were derived from the observed plasma concentration data, while AUC (area under the plasma concentration *vs.* time curve) parameters $AUC_{0–60}$ and $AUC_{0\text{-last}}$ were assessed by integration of the plasma concentration *vs.* time curve using linear interpolation for increasing plasma levels and logarithmic interpolation for decreasing plasma levels (linear up/log down method). PK parameters were calculated for both unadjusted and baseline-adjusted plasma concentration data. $AUC_{0\text{-last}}$ was calculated from time 0 to the time of the last detectable plasma concentration. $AUC_{0–60}$ is the AUC truncated at 60 min. All calculations were based on actual sampling times recorded during the study. Concentrations below the lower limit of quantification (LLOQ) occurring before C_{max} were treated as zero. Concentrations below LLOQ occurring after C_{max} were omitted from the analysis.

Nicotine extraction

The residual nicotine content of the used Nordic Spirit and LD snus pouches was determined by Lablytica Life Science AB (Uppsala, Sweden) using ultra-performance liquid chromatography coupled with ultraviolet detection (UPLC-UV) after extraction using 1:1 methanol–water. This analytical method has been validated according to "Nonclinical Dose Formulation Analysis Method Validation and Sample Analysis, 2010 (4 December 2010, AAPS Journal)" and "A Global GLP approach to Formulation Analysis Method Validation and Sample Analysis, 2011 (ISSN 2153–2435 PAA). Unused Nordic Spirit and LD snus pouches served to determine baseline nicotine content. The difference between the residual nicotine content in the used pouches and the baseline nicotine content in the reference pouches was used to calculate the extracted nicotine amounts.

Subjective measures questionnaires

At the end of each 30 min product use session, the participants filled out a modified product evaluation scale (modified PES) paper questionnaire to subjectively report their product use experience. This questionnaire was composed of the items 1 to 12 of the PES (Product Evaluation Scale). The PES (Hatsukami et al. 2013) is a modified consumer reported outcome measures questionnaire for oral nicotine products adapted from the modified cigarette evaluation questionnaire (Cappelleri et al. 2007).

The modified PES questionnaire contained self-evaluation questions for five subscales covering Product Satisfaction (Was it satisfying?, Did it taste good?, Did you enjoy it?), Psychological reward (Did it calm you down?; Did it make you feel more awake?; Did it make you feel less irritable?; Did it help you concentrate?; Did it reduce your hunger for food?), Aversion (Did it make you dizzy?; Did it make you nauseous?), Craving reduction (Did it immediately relieve your craving for a tobacco product?) and Enjoyment of mouth sensations (Did you enjoy the sensations in your mouth?). Each question was rated on a 7-point graded scale where one corresponded to "not at all" and seven to "extremely".

A visual analog scale (VAS) questionnaire was used to assess the intent to use the product again 8 h after the start of each product use. The question "If given the opportunity, would you use this product again?" was answered with a 100 mm VAS scale printed on a sheet of paper anchored with "Definitely would not" at 0 mm and "Definitely would" at 100 mm. The participants placed a vertical mark anywhere on the 0–100 mm line to reflect their intent to use the product again.

Safety assessments

Adverse events (AEs), including serious adverse events (SAEs), self-reported by the participants, observed by the study medical personnel, or elicited from the participants based on non-leading questions, were collected from the first use of study product until the end of the study. An AE was defined as any untoward medical occurrence in a study participant that used a study product, and which did not necessarily have a causal relationship with this study product (European Medicines Agency 1995). AEs were graded for severity (grade 1 to grade 5) following the common terminology criteria for AEs (CTCAE) v5.0 and assessed as unlikely, possibly, or probably related to the study products. Vital signs (systolic/diastolic blood pressure and pulse rate) were measured prior to each product use as well as at 5 min, 30 min and 1 h after the start of product use. 12-lead electrocardiograms (ECGs) were recorded prior to each product use. Safety assessments carried out after the last product use and prior to discharge from the clinical site included vital signs and ECG measurements, physical examination, blood and urine sampling for standard care clinical chemistry and hematology laboratory parameters, and the collection of AEs and uses of concomitant medication.

Statistical analyses

The number of study participants was considered sufficient for this exploratory study based on similar study designs published in the literature (Rensch et al. 2021; Liu et al. 2022; Chapman et al. 2022). Descriptive summaries and statistical analyses were performed using SAS Version 9.4 (SAS Institute, Inc., Cary NC, United States). Statistical comparisons of each PK parameter were made between each of the Nordic Spirit NPs and the two comparator products. Log transformed nicotine C_{max} and $AUC_{0\text{-last}}$ estimates were evaluated separately in a linear mixed-effects analysis of variance (ANOVA) model with fixed effects for period, sequence, and product, and a random effect for participant. The above product differences were back transformed to present the ratios of geometric least squares (LS) means and 95% confidence intervals (CIs) of each test product versus each comparator product from the same model. The nicotine T_{max} was analyzed separately for each of the above-mentioned product comparisons using a Wilcoxon signed-rank test. Estimates of the median difference based on the observed medians, 95% CIs, and p-values from the Wilcoxon signed-rank test were calculated. Linear regression analysis was performed for assessing the relationship between the extracted amount of nicotine and PK parameters (C_{max} and AUC) with calculating Pearson correlation coefficient and the p value.

Results

Participant demographics

An overview of participant disposition is provided in Suppl. Figure 1. Baseline characteristics and demographics of the study participants are summarized in Table 1. Thirty (30) of the 67 screened participants were enrolled in the study and used all study products as planned. There were 27 males (90%) and three females (10%), with a mean age of 32.3 years (SD = 10.5) and a mean BMI of 24.2 kg/m^2 (SD = 3.2). Except for one, all participants identified as not Hispanic or Latino. The participants had used snus products for a mean of 10.6 years (SD = 8.7), using on average 15.3 snus pouches per day (SD = 6.7) with a mean nicotine content of 10.42 mg nicotine per pouch (SD = 1.80).

Nicotine pharmacokinetics

The mean plasma nicotine concentration over time curves for each study product are shown in Fig. 2. The shapes of the curves were similar for all study products. A descriptive summary of the nicotine PK parameters is reported in Table 2. Although unadjusted and baseline-adjusted nicotine PK parameters were calculated, similar trends were observed between the two datasets (Suppl. Table 1), therefore only unadjusted data are presented.

As expected, an increase in the nicotine content of the Nordic Spirit NPs resulted in an increased mean maximum nicotine concentration (C_{max}), with a trend toward dose proportionality: 6 mg, 10.92 ng/mL; 9 mg, 13.81 ng/mL; and 11.2 mg, 17.33 ng/mL). The median time to reach C_{max} was 30 min for the 6 mg and 11.2 mg Nordic Spirit NPs and 38 min for the 9 mg NP formulation. The extent of nicotine absorption (AUC) also followed this trend. Mean $AUC_{0–60}$ values were 8.56, 10.55 and 12.90 h*ng/mL, and mean $AUC_{0\text{-last}}$ values were 33.19, 41.18 and 51.77 h*ng/mL, for the 6 mg, 9 mg, and 11.2 mg Nordic Spirit NPs, respectively. For the comparator products, the mean C_{max} for LD snus (11.2 mg) was 8.18 ng/mL and 9.23 ng/mL for Nicorette® gum which were reached at 45 min (T_{max}) as median time for both study products. The mean $AUC_{0–60}$ and $AUC_{0\text{-last}}$ values

Table 1 Baseline characteristics and demographics of study participants

Parameter (unit)		N = 30
Age (years)	Mean (SD)	32.3 (10.5)
Sex	Female n (%)	3 (10%)
	Male n (%)	27 (90%)
Ethnicity	Hispanic or Latino n (%)	1 (3.3%)
	Not Hispanic or Latino n (%)	29 (97%)
Height (cm)	Mean (SD)	178.9 (7.0)
Weight (kg)	Mean (SD)	77.5 (11.8)
BMI (kg/m^2)	Mean (SD)	24.2 (3.2)
Number of snus used per day	Mean (SD)	15.3 (6.7)
Number of years of snus use	Mean (SD)	10.6 (8.7)
Nicotine contents of participants' usual brands of snus (mg/pouch)	Mean (SD)	10.42 (1.80)

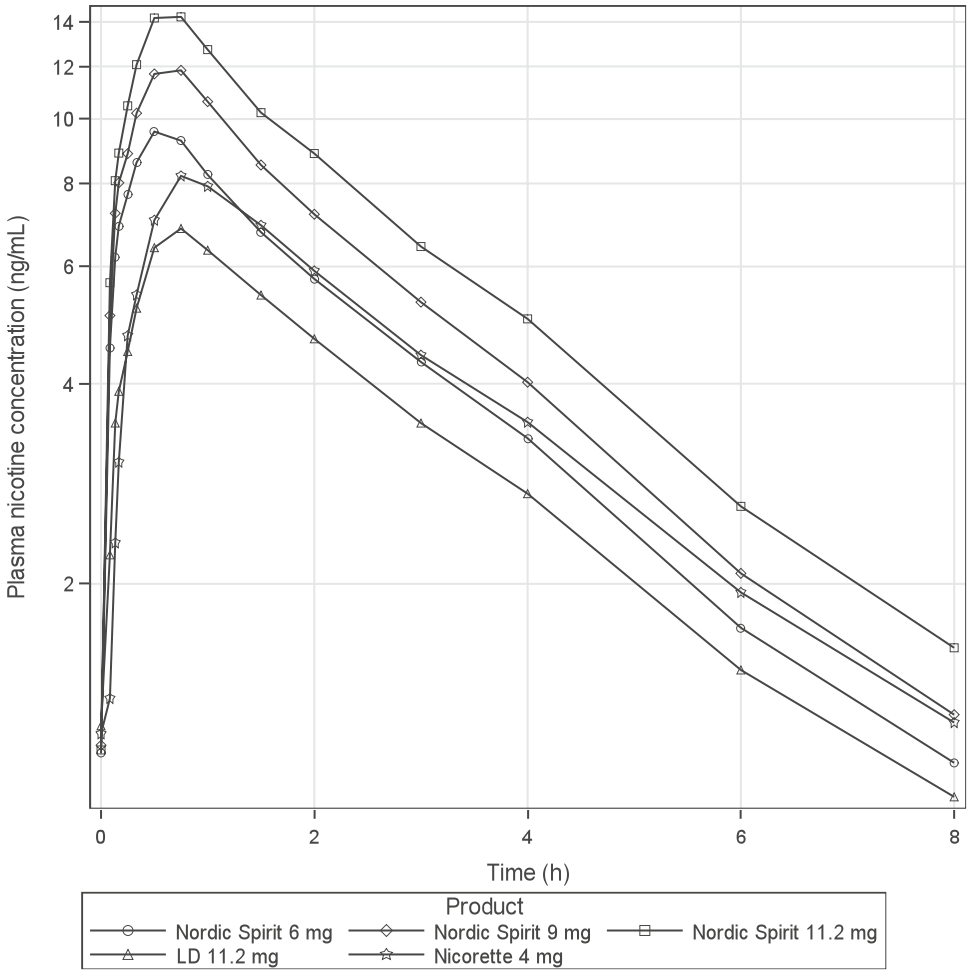

Fig. 2 Mean unadjusted nicotine plasma concentrations (logarithmic scale) per product (N = 30)

Table 2 Summary of PK parameters. Based on unadjusted nicotine plasma levels. N = 30 participants

Assessment (unit)		Nordic Spirit 6 mg	Nordic Spirit 9 mg	Nordic Spirit 11.2 mg	LD 11.2 mg	Nicorette® 4 mg
T_{max} (h)	Median (Min, Max)	0.50 (0.13, 1.00)	0.63 (0.08, 1.00)	0.50 (0.14, 1.50)	0.75 (0.17, 2.00)	0.75 (0.50, 1.50)
C_{max} (ng/mL)	Mean (95% CI)	10.92 (9.49, 12.34)	13.81 (11.85, 15.76)	17.33 (14.28, 20.37)	8.18 (6.37, 9.99)	9.23 (8.08, 10.37)
	Geometric mean (CV%)	10.34 (33.7%)	13.03 (34.0%)	15.81 (44.1%)	7.35 (45.3%)	8.63 (42.7%)
AUC_{0-60} (h*ng/mL)	Mean (95% CI)	8.56 (7.51, 9.61)	10.55 (9.20, 11.90)	12.90 (10.81, 15.00)	5.97 (4.90, 7.04)	6.45 (5.61, 7.30)
	Geometric mean (CV%)	8.17 (31.6%)	10.05 (31.6%)	11.96 (39.5%)	5.51 (39.7%)	8.17 (31.6%)
AUC_{0-last} (h*ng/mL)	Mean (95% CI)	33.19 (29.05, 37.32)	41.18 (35.42, 46.94)	51.77 (42.77, 60.77)	27.87 (20.75, 34.98)	32.78 (28.21, 37.35)
	Geometric mean (CV%)	31.65 (31.2%)	38.99 (33.1%)	47.69 (40.8%)	24.66 (47.3%)	30.35 (45.9%)

AUC = area under the nicotine plasma concentration *vs.* time curve, AUC_{0-60} = AUC from time 0 to 60 min, AUC_{0-last} = AUC from time 0 to the last time point with a measurable nicotine plasma concentration, C_{max} = maximum observed plasma nicotine concentration, CV = coefficient of variance, T_{max} = time of occurrence of C_{max}

were 5.97 and 6.45 h*ng/mL and 27.87 and 32.78 h*ng/mL for the LD snus and the Nicorette® gum comparators, respectively. Moderate to high positive correlations of the extracted amount of nicotine with either C_{max} or $AUC_{0\text{-last}}$ were observed for Nordic Spirit NPs and LD snus (Suppl. Table 3).

A summary of the statistical comparisons of C_{max} and $AUC_{0\text{-last}}$ is shown in Table 3. The mean C_{max} of each of the Nordic Spirit NPs was significantly higher ($p < 0.05$) than those of both LD snus and the Nicorette® gum. Moreover, the mean $AUC_{0\text{-last}}$ for each of the Nordic Spirit NPs was also significantly higher ($p < 0.05$) than the mean $AUC_{0\text{-last}}$ of LD snus and Nicorette® gum except for Nordic Spirit NP 6 mg and Nicorette® gum which was not statistically significant different.

Subjective measures

Subjective measures results are summarized in Table 4. For the modified PES questionnaire, the mean scores were calculated for the subscales Satisfaction, Psychological reward, Aversion, Craving reduction, and Enjoyment of mouth sensation. For all subscales, the mean scores in the three Nordic Spirit NPs use were similar based on overlaps of the 95% CIs, except that the "Aversion" score in Nordic Spirit 6 mg use was lower than those in Nordic Spirit 9 and 11.2 mg without overlaps of 95% CI. For all subscales, the mean scores in LD snus use were generally similar with those in the three Nordic Spirit NPs use based on overlaps of the 95% CIs. For all subscales except the Aversion, the mean scores in Nicorette® gum use were lower than those in the other

Table 3 Statistical comparisons of selected PK parameters

PK variable	Geometric mean		Ratio of geometric mean (95% CI)	p-value
	Nordic Spirit	Reference product		
$AUC_{0\text{-last}}$ (h*ng/mL)	Nordic Spirit 6 mg	LD Original Vit Stark 11.2 mg	1.3 (1.1, 1.5)	0.0002
	Nordic Spirit 6 mg	Nicorette®, mint flavor gum 4 mg	1.0 (0.9, 1.2)	0.5133
	Nordic Spirit 9 mg	LD Original Vit Stark 11.2 mg	1.6 (1.4, 1.8)	< .0001
	Nordic Spirit 9 mg	Nicorette®, mint flavor gum 4 mg	1.3 (1.1, 1.5)	0.0002
	Nordic Spirit 11.2 mg	LD Original Vit Stark 11.2 mg	1.9 (1.7, 2.2)	< .0001
	Nordic Spirit 11.2 mg	Nicorette®, mint flavor gum 4 mg	1.6 (1.4, 1.8)	< .0001
C_{max} (ng/mL)	Nordic Spirit 6 mg	LD Original Vit Stark 11.2 mg	1.4 (1.2, 1.6)	< .0001
	Nordic Spirit 6 mg	Nicorette®, mint flavor gum 4 mg	1.2 (1.1, 1.3)	0.0036
	Nordic Spirit 9 mg	LD Original Vit Stark 11.2 mg	1.8 (1.6, 2.0)	< .0001
	Nordic Spirit 9 mg	Nicorette®, mint flavor gum 4 mg	1.5 (1.3, 1.7)	< .0001
	Nordic Spirit 11.2 mg	LD Original Vit Stark 11.2 mg	2.2 (1.9, 2.4)	< .0001
	Nordic Spirit 11.2 mg	Nicorette®, mint flavor gum 4 mg	1.8 (1.6, 2.0)	< .0001

AUC = area under the nicotine plasma concentration vs. time curve, $AUC_{0\ last}$ = AUC from time 0 to the last time point with a measurable nicotine plasma concentration, CI = confidence interval, C_{max} = maximum observed plasma nicotine concentration

Table 4 Summary of subjective measures. Modified PES and VAS subscore means with 95% CI lower and upper bounds. N = 30

Question		Nordic Spirit 6 mg	Nordic Spirit 9 mg	Nordic Spirit 11.2 mg	LD 11.2 mg	Nicorette® 4 mg
Satisfaction	Mean (95% CI bounds)	4.64 (4.21, 5.08)	5.04 (4.70, 5.38)	5.02 (4.72, 5.32)	4.85 (4.36, 5.35)	3.21 (2.60, 3.81)
Psychological reward	Mean (95% CI bounds)	4.20 (3.80, 4.60)	4.52 (4.23, 4.81)	4.60 (4.20, 5.00)	4.09 (3.71, 4.48)	3.69 (3.21, 4.18)
Aversion	Mean (95% CI bounds)	1.07 (0.96, 1.17)	1.33 (1.12, 1.55)	1.43 (1.19, 1.68)	1.27 (1.07, 1.47)	1.83 (1.35, 2.32)
Craving reduction	Mean (95% CI bounds)	4.47 (3.91, 5.03)	4.97 (4.44, 5.49)	5.10 (4.58, 5.62)	5.40 (4.95, 5.85)	3.33 (2.70, 3.97)
Enjoyment of mouth sensations	Mean (95% CI bounds)	4.93 (4.37;5.50)	5.20 (4.87;5.53)	4.97 (4.53;5.40)	4.97 (4.37;5.57)	3.07 (2.43;3.70)
Intent to use the product again (VAS)	Mean (95% CI bounds)	49.2 (39.1;59.3)	61.3 (53.3;69.4)	60.7 (52.0;69.4)	60.7 (50.1;71.3)	23.4 (12.6;34.3)

study products use. Such lower mean scores in Nicorette® gum use was evident for the Satisfaction and the Enjoyment of mouth sensation due to non-overlap of the 95% CI with the other study products use. The similar tendency was also observed for the mean scores of the Craving reduction, though the 95% CIs were overlapped between Nicorette® gum and Nordic Spirit 6 mg. For the Aversion, the mean score in Nicorette® gum use was similar with those in the other study product use with overlap of the 95% CI, except that the mean score was higher in Nicorette® gum use than in Nordic Spirit 6 mg use.

In addition, intention to use the product again was measured with a VAS. The mean scores in the three Nordic Spirit NPs use were similar based on overlaps of the 95% CI. In addition, the mean score in LD snus use was similar with those in Nordic Spirit NPs use based on overlaps of the 95% CIs. The mean score in Nicorette® gum use was lower than those in the other study products use, without overlaps of the 95% CIs. The mean scores shows that subjects' intention to use the product again was neutral for Nordic Spirit 6 mg, positive for Nordic Spirit 9 and 11.2 mg and LD snus, and negative for Nicorette® gum.

Physiological effects

Changes of vital signs including blood pressure and pulse rate were measured from baseline up to one hour after start of study products use, i.e., up to 30 min after end of the products use (Suppl. Table 2).

At the baseline, no noticeable difference in the vital signs levels was observed among each session of the study products use. As expected, nicotine is a mild stimulant of the sympathetic nervous system, for all study product, the vital sign levels were slightly increased during 30 min of the products use, with the mean values ranging from 3.2 to 6.0% for systolic blood pressure, from 6.7 to 11.3% for diastolic blood pressure and 3.0 to 10.9% for pulse rate. At one hour after start of study products use, relative change from baseline (%) of systolic blood pressure were −0.1, 3.0, 1.4, 0.7 and 2.6 in Nordic Spirit 6 mg, 9 mg, 11.2 mg, LD snus and Nicorette® gum use, respectively. The % change of diastolic blood pressure were 4.5, 7.4, 3.9, 5.7 and 8.2 in Nordic Spirit 6 mg, 9 mg, 11.2 mg, LD snus and Nicorette® gum use, respectively. The % change of pulse rate were −3.0, 3.2, 0.2, −3.1, 1.8 in Nordic Spirit 6 mg, 9 mg, 11.2 mg, LD snus and Nicorette® gum use, respectively. Overall, at one hour after start of study products use (30 min after end of study product use), the measured vital sign levels were not substantially different from the baseline levels indicating the transient effects. Moreover, the % changes of vital signs were not markedly different among the three Nordic Spirit NPs with varying nicotine content. The % changes of vital signs in Nordic Spirit NPs use were also not notably different from those in the LD snus and Nicorette® gum use.

Safety aspects

Adverse events

Five (5) out of the 30 enrolled participants (17%) reported 9 AEs in total. There were no SAEs reported, and no study discontinuations due to AEs. All AEs were mild in intensity and resolved within one day or less. The most reported AEs were headache (3 events) and hiccups (2 events), both transient and self-limiting events known and commonly observed with nicotine product use. Five (5) of the AEs (56%) were assessed as possibly related to the study products, mostly to the Nicorette® gum (3 AEs, hiccups, nausea, and dyspepsia). One AE (headache) was assessed as possibly related to Nordic Spirit 6 mg, and one (hiccups) was assessed as possibly related to LD snus 11.2 mg. For the remaining 4 AEs (44%) the causality assessment was reported as unlikely or not applicable.

There were no differences in AE reporting frequency between the Nordic Spirit NPs and the comparators, irrespective of nicotine content.

Additional safety measures

ECGs, clinical chemistry, hematology, and urinalysis parameters as well as physical examination findings assessed after the last product use were within normal ranges, and no changes from baseline measurements were considered clinically significant.

Discussion

This randomized clinical study aimed to characterize the nicotine PK after a single use of three Nordic Spirit NPs (6 mg, 9 mg and 11.2 mg/pouch) following 30 min of product use. LD tobacco snus (11.2 mg/pouch) and Nicorette® gum (4 mg/unit) were used as oral nicotine delivery comparators. LD snus was selected since this traditional tobacco product for oral use shares similarities with Nordic Spirit NPs in terms of the product shape, usage, and nicotine contents (11.2 mg per pouch). Nicorette® gum was selected as this tobacco-free nicotine-containing product for oral use shares similarities with Nordic Spirit NPs in terms of the formulation (nicotine Polacrilex and gum base as the major ingredients). As supportive information to assess the impact of Nordic Spirit on human use, subjective and physiological effects were characterized, and safety monitoring was conducted during the study period.

No strict control was performed for proportion of sex, therefore, the male and female proportions were not the same in the present study and likely reflected the demographic tendency among Swedish snus users. While it cannot completely rule out the possibility that an imbalance in the proportion of sex may have impacted interpretations, no evident intersubject-differences were observed for the data in relation to difference in sex.

The obtained nicotine PK curves following use of the study products demonstrated single nicotine peaks for each product type, with PK parameters for the Nordic Spirit NPs within the range of those reported previously for NPs in the literature (Lunell et al. 2020; McEwan et al. 2022; Liu et al. 2022). As expected, and consistent with the broader scientific literature, variations in the nicotine content of the NP had an impact on nicotine delivery. With increasing nicotine content in the NPs, C_{max} and $AUC_{0\text{-last}}$ increased, with a trend toward dose proportionality, and the C_{max} and $AUC_{0\text{-last}}$ also showed positive correlation with the measured extracted amount of nicotine.

The nicotine PK parameter data for Nicorette® gum was also consistent with those reported in the literature (Lunell and Curvall 2011; Lunell et al. 2020; Azzopardi et al. 2022a). Nicorette® gum, which has lower nicotine content (4 mg per piece) and similar composition to the Nordic Spirit NPs, showed slightly lower mean C_{max} and $AUC_{0\text{-last}}$ compared to Nordic Spirit 6 mg use, aligning with the dose response relationship for nicotine-containing products. The nicotine PK for the LD snus showed significantly lower C_{max} and $AUC_{0\text{-last}}$ than those for Nordic Spirit NPs, including the 11.2 mg/pouch NP formulation which had the same nicotine content as the LD snus. This observation is consistent with the clinical findings from Lunell et al. 2020 that showed a 6 mg NP formulation had a greater blood nicotine delivery profile compared to an 8 mg tobacco snus comparator. This might be attributed to the differences in product characteristics such as moisture and pH, as well as the nicotine-containing matrix used. These factors may have influenced the efficiency of blood nicotine delivery within the buccal cavity as previously documented (Aldeek et al. 2021).

The median T_{max} values for the Nordic Spirit NPs were shorter compared to the LD snus and Nicorette® gum comparators (NP, 30–38 min; LD snus and Nicorette® gum, 45 min), which is broadly correlated with end of NP use (30 min). For the Nordic Spirit NPs, the observation that T_{max} is reached immediately after end of use is consistent with findings from other NP clinical studies that show a good correlation between product usage time and the time the C_{max} is reached (Lunell et al. 2020; Liu et al. 2022; Chapman et al. 2022). However, for both LD snus and Nicorette® gum the T_{max} was observed sometime after the end of product use. This observation is consistent with results in the published literature (Lunell and Curvall 2011; Azzopardi et al. 2022a). Interestingly, the T_{max} for LD snus seemed to be slightly delayed. The nicotine delivery from the NP product stopped immediately when the user ceased to use it, unlike the extended release observed with snus and Nicorette® gum. This could be due to the filler matrix. The nicotine in Nordic Spirit NPs is in a powder form within the filler materials, while in LD snus it is in tobacco leaves, and in Nicorette® gum, it is part of a solid gum base.

The observed characteristics of Nordic Spirit NPs described above are favorable for profiling NP nicotine delivery based on nicotine content, including particularly for profiling safety in human use and further product developments. Notably, real-world use duration of NPs differs between users. Since T_{max} in Nordic Spirit NPs use was detected immediately after end of use, use duration data from real-world use could be utilized to estimate the timing to reach maximum plasma nicotine concentrations.

Subjective effects assessment was used as an exploratory method to evaluate user experience with the study product in participants who were exclusive Swedish snus users. For all modified PES subscales, the mean scores after Nordic Spirit NPs use were similar based on the overlaps of the 95% CIs, except that the Aversion score after Nordic Spirit 6 mg use was lower than for the other Nordic Spirit NPs. These scores after Nordic Spirit NPs use were similar to those observed for the LD snus use. Similarly, no substantial difference was observed for the intention to use the product again score between the Nordic Spirit NPs use and the LD snus. The mean intention to use the product again scores were positive for the Nordic Spirit 9 and 11.2 mg and LD snus use but neutral after Nordic Spirit 6 mg use. Overall, the current findings revealed no evident difference between the Nordic Spirit NPs and LD snus products in terms the user experience, both for positive sensations (Satisfaction, Psychological reward, Craving reduction, and Enjoyment of mouth sensation) and negative sensations (Aversion), as well as intention to use the product again. Interestingly, even though there were overlaps of 95% CIs for Nordic Spirit NPs and LD snus, the values for 'satisfaction' and 'Intent to use product again' were slightly lower following the use of Nordic Spirit 6 mg.

Mean subjective measure scores after Nicorette® gum use tended to be different compared to after Nordic Spirit NPs and LD snus use, especially regarding the lower Satisfaction and Enjoyment of mouth sensation scores of modified PES, as well as the negative intention to use the product again score. Since the highest mean Aversion score was observed for the Nicorette® gum use, such stronger negative experience on the product use might be more predominant than the other factors. Liu et al. 2022 reported that nicotine delivery and subjective effects in NPs use were not likely to be higher than smokeless tobacco products, which is consistent with the findings of the present study.

Physiological effects assessment and monitoring of safety were also performed in the present study. Nicotine is known to cause transient alterations to cardiovascular parameters through the activation of the sympathetic nervous system (Price and Martinez 2019). Therefore, the short-term cardiovascular impacts of the study products were measured. As expected, blood pressure and pulse rate increased during study product use, with both measures returning to baseline levels 30 min after end of use. No dose-dependent response relationship was observed among the three Nordic Spirit NPs. Changes to the physiological measure levels after Nordic Spirit NPs use were also no different from those after LD snus and Nicorette® gum comparator uses. These findings suggest that differences in nicotine concentration and product formulation did not significantly alter the transient cardiovascular response to the test products.

No serious AEs were observed, and only mild AEs were reported in the present study. The AEs possibly related to the use of the study products were single events of headache (Nordic Spirit 6 mg use), hiccups (LD snus and Nicorette® gum use) as well as nausea, and dyspepsia (Nicorette® gum use). These are commonly observed, transient and self-limiting effects of nicotine product use that have been well-documented in the scientific literature. No clinically significant findings were observed for the safety-relevant measurements including ECGs, clinical chemistry, hematology and urinalysis parameters, as well as physical examinations. Overall, no safety concerns were raised after single use of the study products in the present study.

Limitations

This study has several limitations, and the results should be viewed within this context. This was a single use, controlled study in a clinical setting and so results may not be fully reflective of real-world product use behaviors and preferences. Assessments under ad libitum study product use condition would be of value for further understanding of the health effects of NPs in a real-world situation.

Daily Swedish snus users were enrolled in this study, as they were assumed to be familiar with tobacco/nicotine-containing products for oral use. However, no period of adaptation to the novel products was included for the participants, which may have had an impact on some of the outcomes, particularly the subjective measures. In addition, combustible cigarette smokers, a major population of conventional tobacco users, were not included in the present study. Understanding use across different types of tobacco consumers would be of value for estimating the impacts of NPs in future studies. In addition, no strict control was performed for proportion of sex, therefore, the male and female proportions were not the same in the present study and likely reflected the demographic tendency among Swedish snus users. While it cannot completely rule out the possibility that an imbalance in the proportion of sex may have impacted interpretations, no evident intersubject-differences were observed for the data in relation to difference in sex.

Conclusions

Delivery of nicotine from the three Nordic Spirit NPs appeared to be nicotine content-dependent, based on C_{max} and AUC. Moreover, the amount of nicotine extracted following oral use showed positive correlation with the reported C_{max} and AUC for the Nordic Spirit NPs. T_{max} in Nordic Spirit NPs use were detected immediately after end of use. As a whole, the characteristics of Nordic Spirit NPs were found to be favourable for profiling NP nicotine delivery and safety in human use, as well as for further product development. Overall, no notable safety concerns were observed after single use for any of the study products, including no significant adverse effects.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00213-024-06721-7.

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Declarations

The study was conducted by CTC Clinical Trial Consultants AB under contract to JTI in accordance with ethical principles that have their origin in the Declaration of Helsinki, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6 (R2) guideline for Good Clinical Practice (GCP), as well as the European Union (EU) Clinical Trials Directive 2001/20/EC. The participants' personal data was protected in accordance with the EU General Data Protection Regulation (GDPR) 2016/679. The study was approved by a competent authority (Swedish Medical Products agency approval no. 5.1–2020-60886) and an independent ethics committee (Swedish Ethical Review Authority approval no. 2020–05745) prior to study initiation. All participants in the study reviewed, signed, and dated an informed consent form prior to study initiation.

Conflict of interest During the study, D.N. was employed by Japan Tobacco Inc., and K.R., S.L., L.G. and J.M. were employed by JT International SA. J.N. was employed by CTC Clinical Trial Consultants AB and was the Principal Investigator of the study.

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