Clinical Study Protocol

Investigational products	Nicotine pouches
Study code	SM24-01
Protocol version and date	FINAL v2.0; 09OCT2024

Examining the Effects of Nicotine Content in Nicotine Pouches on Nicotine Exposure Under Controlled Settings and During *Ad Libitum* Use: A Comprehensive Three-Part Study.

Test products and dose	Nicotine pouch (NP) 1 - 6 mg nicotine/pouch (Part 2, and option in Part 3) NP 2 - 11 mg nicotine/pouch (Part 2, and option in Part 3) NP 3 – 16.6 mg nicotine/pouch (Part 2, and option in Part 3)
Comparator products and dose	Subject's own brand snus product, pouched or loose snus portion (Part 1 and Part 2) Swedish pouch snus (General G.3 Extra Strong Slim), 18 mg nicotine/pouch (Part 2)
Sponsor signatory	[redacted] Swedish Match Maria Skolgata 83 SE-118 53 Stockholm, Sweden [redacted]
Coordinating Investigator	Johan Nilsson, MD, PhD Clinical Trial Consultants AB (CTC)
Clinical study management	Clinical Trial Consultants AB (CTC) Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden

DOCUMENT HISTORY

The following modifications have been made to the first approved version of this Clinical Study Protocol (version 1.0).

Type of change	Date of amendment	Summary of changes	Revised protocol version
Updated version for re-submission.	09OCT2024	• Study title changed from "Examining nicotine limits for nicotine pouches: a comprehensive three-part study" to "Examining the effects of nicotine content in nicotine pouches on nicotine exposure under controlled settings and during *ad libitum* use: a comprehensive three-part study". • NP strengths adjusted from 8, 12 and 16 mg to 6, 11 and 16.6 mg. • Clarification of study background and study rational. • Updated risk-benefit assessment. • Changed inclusion requirement to a minimum daily snus consumption of 5 pouches/portions. • Addition of Fagerström test for nicotine dependence to confirm the participant's level of nicotine dependence. • Study time period adjusted.	V2.0_09OCT2024

1 STUDY SYNOPSIS

Study title

Examining the Effects of Nicotine Content in Nicotine Pouches on Nicotine Exposure Under Controlled Settings and During *Ad Libitum* Use: A Comprehensive Three-Part Study.

Study code	**Planned study period**
SM24-01	Q1 2025 to Q4 2025

Coordinating/Principal Investigator

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Study design

This is a multi-center, open-label, three-part study:

- *Part 1:* a multi-center, non-randomized observational part involving use of the subject´s own brand Swedish snus product of choice (hereafter, called own brand snus) *ad libitum.*
- *Part 2*: an open-label, multi-center, randomized, 5-way cross-over, single use part. The investigational products (IPs) include three moist nicotine pouch (NP) products, containing 6 mg/unit, 11 mg/unit, and 16.6 mg/unit of nicotine. The comparator products include one Swedish snus 18 mg/unit product and subject´s own brand snus product.
- *Part 3:* a multi-center, non-randomized interventional part involving complete substitution of own brand snus product with a NP product for *ad libitum* use.

Objectives and endpoints

Primary objective	**Endpoint**
To demonstrate that the moist nicotine pouch (NP) 11 mg product does not result in substantially higher nicotine exposure compared to the comparator product Swedish snus 18 mg.	To compare nicotine exposure, as measured by baseline-adjusted area under the plasma concentration *vs.* time curve from 0 to infinity ($AUC_{0\text{-}inf}$) based on nicotine plasma concentrations, between the moist NP 11 mg product and the comparator product, Swedish snus 18 mg. (The goal is to demonstrate that the upper bound of the 95 % confidence interval for the ratio for nicotine exposure between the moist NP 11 mg product and the comparator product is at or below 1.25.)

No.	**Secondary objectives**	**No.**	**Endpoints**
1.	To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	1.	The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.
2.	To compare the pharmacokinetic (PK) profile between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products,	2a.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, in the non-adjusted and baseline-adjusted PK parameters based on plasma concentrations of nicotine:

	Swedish snus 18 mg and own brand snus product.		$AUC_{0\text{-}inf}$, maximum observed plasma concentration (C_{max}), time to C_{max} (T_{max}), AUC from 0 to 1.5 hours ($AUC_{0\text{-}1.5h}$), AUC from 0 to time of last measurable time point ($AUC_{0\text{-}last}$), and terminal elimination half-life ($T_{1/2}$).
		2b.	To compare nicotine exposure, as measured by non-adjusted and baseline-adjusted $AUC_{0\text{-}inf}$, between the moist NP 6 mg and NP 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product. This comparison also includes the NP 11 mg product and the own brand snus product. (The goal is to demonstrate that the upper bound of the 95 % confidence interval for the ratio for nicotine exposure between the moist NP products and the comparator products is at or below 1.25.)
3.	To assess the pharmacodynamic (PD) effects, measured as pulse rate and subjective outcome measures, of the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	3a.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, for the highest recorded increase (E_{max}), the time to E_{max} (T_{Emax}), the E_{max} from time 0 to 60 minutes ($E_{max0\text{-}60}$), and the time to reach $E_{max0\text{-}60}$ ($T_{Emax0\text{-}60}$) in pulse rate from baseline, measured using a pulse oximeter after IP use.
		3b.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, for the largest recorded decrease (E_{max}) in the subjective parameter "craving" and the highest recorded value for "satisfaction", measured using a 100 mm visual analog scale (VAS) after IP use.
		3c.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 30 minutes after IP use.
4.	To evaluate the safety and tolerability of the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	4.	Frequency, intensity, and seriousness of adverse events (AEs).

No.	**Exploratory objectives**	**No.**	**Endpoints[1]**
1.	To evaluate the impact on "product-liking" *vs.* the subjects' usual Swedish snus product of choice by administering single doses of the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator product, Swedish snus 18 mg.	1.	Difference in "product-liking" *vs.* the subjects' usual Swedish snus product of choice between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator product, Swedish snus 18 mg, measured on a 3-point scale using a multiple-choice question (MCQ) 30 minutes after IP use.
2.	To compare the nicotine extraction normalized PK parameters $AUC_{0\text{-}inf}$ and C_{max} between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	2.	Difference in nicotine extraction normalized $AUC_{0\text{-}inf}$ and C_{max} between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.
3.	To analyze the pattern of use during each of the two 14-day *ad*	3.	Difference in the pattern of use during each of the two 14-day *ad libitum* usage periods of either own

	libitum usage periods of either own brand snus product usage or complete substitution with a moist NP product.		brand snus product usage or complete substitution with a moist NP product.
4.	To compare the PK profile between the selected strength NP product and the usual brand snus product.	4.	To compare nicotine exposure (in Part 2), as measured by baseline-adjusted $AUC_{0\text{-}inf}$ and C_{max}, between the selected strength NP product (in Part 3) and the usual brand snus product (in Part 1).
5.	To compare urine concentrations and amounts of biomarkers of exposure (BoE) (nicotine and its metabolites, N -nitrosonornicotine [NNN], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL], and anabasine) before and after complete substitution of own brand snus product with a moist NP product for 14 days.	5.	Difference in creatinine-normalized urine concentrations and amounts of BoE (nicotine, and its metabolites, NNN, NNAL, and anabasine) before and after complete substitution of own brand snus product with a moist NP product for 14 days.
6.	To compare the extracted amounts and fractions of nicotine, NNN, and NNK from used and collected own brand snus pouches/portions and used moist NPs.	6.	Difference in the extracted amounts (mg/unit) and fractions (%) of nicotine, NNN, and NNK from used and collected pouches/portions during each of the two 14-day *ad libitum* usage periods of either own brand snus product usage or complete substitution with a moist NP product.
7.	To correlate the extracted amounts of nicotine, NNN, and NNK, multiplied by the number of pouches/portions used per day on average, with urine concentrations of BoE.	7.	The correlation between the extracted amounts (mg/unit) of nicotine, NNN, and NNK, multiplied by the number of pouches/portions used per day on average, and the creatinine-normalized urine concentrations of BoE before and after complete substitution of the own brand snus product with a moist NP product for 14 days.

1. Loose snus users will be analyzed as a subgroup for all exploratory endpoints.

Number of subjects planned

The study will include approximately 53 tobacco-based snus users with the aim of randomizing at least 53 subjects in Part 2 and achieving 45 evaluable subjects (for Part 2).

Efforts will be made to include at least 21 female subjects (approximately 40%). However, a minimum of 11 female subjects (approximately 20%) will be considered acceptable.

Additionally, efforts will be made to include 10 to 15 regular users of loose snus. These subjects will use a loose snus of their choice as their own brand product during both Part 1 and Part 2.

Diagnosis and main eligibility criteria

Healthy male or female subjects aged ≥19 to ≤60 years who have used Swedish snus products for ≥1 year, with a minimum daily consumption of 5 pouches/portions, who are willing and able to use both Swedish snus and NPs with high nicotine content, while abstaining from other tobacco/nicotine products during the study, may be considered to be eligible for participation in the study. All subjects must be willing to comply with study procedures and give written informed consent. Female subjects of childbearing potential must practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) or must agree to use a highly effective method of contraception with a failure rate of <1 % to prevent pregnancy for the duration of the study.

Subjects who intend to change their nicotine consumption habit or to stop using nicotine-containing products, and/or subjects who are pregnant, breastfeeding, or intend to become pregnant during the study, and/or subjects with a history or presence of diagnosed hypertension or cardiovascular disease

or other medical condition that may interfere with the evaluation of the IPs or may put the subject at risk because of participation in the study, will be excluded from the study.

Methodology

The screening visit (*Visit 1*) will take place within 5 weeks prior to start of Part 2 (Visit 4) and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, collection of medical history, a brief physical examination, serology tests, electrocardiogram (ECG), vital signs (pulse rate and blood pressure), height, weight, and body mass index (BMI) assessments. During the screening visit, subjects will complete a nicotine dependence questionnaire. They will also select the Swedish snus product, either a pouch or loose snus, which they will use exclusively during Part 1 of the study. Subjects will purchase this product themselves. The chosen product should be the brand they predominantly used in the past month unless they did not exclusively use a single brand. This selection will determine the nicotine strength and flavor variation, as these factors vary within the same brand. For Part 2 of the study and for chemical analyses, the Sponsor will purchase the selected snus product.

Part 1: *Ad libitum* use of own brand snus

At Visit 2 (first day of Part 1), eligible subjects will return to the study site to receive collection materials for used pouches, undergo eDiary training, and undergo interviews regarding their health status and medications used.

After Visit 2, subjects will exclusively use their product of choice *ad libitum*, following their regular pattern of use. They will document their consumption via an eDiary (ViedocMe) during a 14-day study period, recording the number of pouches/portions used per day, the estimated usage time per pouch/portion, and whether they opened a new snus can for pouch/portion usage once per day. Also, subjects will collect 4 used pouches/portions on two separate days the first week (Samples A) and on two separate days the second week (Samples B) and store these in a freezer (≤ -18°C). In total, 8 pouches/portions will be collected per week. Subjects will receive instructions on how to collect used pouches/loose snus and will be provided with collection containers for storing them and a cooling bag for transportation of the used pouches to the study site.

At Visit 3 (14 days [allowed time window: +3days] after Visit 2), the subjects will return to the study site in the morning. They will bring the collected frozen pouches/portions for extraction analysis. During this visit, subjects will undergo interviews regarding their health status and medications used, the specific Swedish snus product they used (including its strength and flavor), and their compliance with the eDiary will be assessed. Additionally, a 24-hour urine collection (allowed time window: ±15 minutes) will begin in the morning. Subject will remain on-site until the following morning, using the snus product *ad libitum*. The urine will be continuously collected during this period for the analysis of biomarkers of exposure (BoE).

Part 2: Single IP use in a 5-way cross-over

At Visit 4, the subjects will return to the study site for the first IP use visit of Part 2.

Subjects shall abstain from tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for 12 hours prior to each IP use visit (Visits 4 to 8). All IP use sessions will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

The IPs will be administered as single pouches/portions in a pre-determined randomized order. Randomization will take place before the first IP use of Part 2.

Subjects will keep the pouch/portion between their lip and gum for 30 minutes and will be instructed not to eat, drink, chew gum, or brush their teeth for 30 minutes before, during, and 30 minutes after the IP use.

After 30 minutes, each used pouch/portion will be collected and frozen (-20°C) pending analysis of residual nicotine content. Unused pouches/portions from the same batch will serve as references and will be stored at -20°C pending analyses.

Blood samples for the assessment of nicotine plasma levels and PK parameters will be collected at pre-defined time points from pre-use to 6 hours after each IP use. The PD effects of the IPs will be determined using pulse rate measurements and subjective parameters (using visual analogue scale [VAS] questions) at the same pre-defined time points as well as a MCQ 30 minutes after IP use.

AEs will be collected through subject interviews and will also include any AEs reported spontaneously by the subjects, starting from the initiation of IP use (Visit 4), and continuing until the last IP use visit (Visit 8).

Visits 5 to 8 will follow the same schedule as Visit 4. Each visit will be scheduled on separate days, preferably with at least 24 hours between visits. However, it is acceptable to have visits on consecutive days, as long as all 5 visits are not scheduled consecutively.

At the end of *Visit 8*, the subject will receive one can of each of the three moist NP products with varying strengths (6 mg, 11 mg, and 16.6 mg) used in Part 2. Importantly, the subjects will be blinded to the nicotine strength of these three products. The subject will test these at home over 2-3 days to ensure they find a suitable replacement for their own brand snus product to be used in Part 3.

Part 3: *Ad libitum* use of nicotine pouch product

At Visit 9 (first day of Part 3), the subjects will return to the study site and then the chosen NP product (6 mg/unit, 11 mg/unit or 16.6 mg/unit) will be documented. Based on the subject's reported consumption, they will be provided with the NP product to be exclusively used *ad libitum* for 14 days (allowed time window: +3 days). During this visit, subjects will undergo interviews regarding any experienced AEs. Subjects will be reminded on how to document their consumption using the eDiary and how to collect used pouches. The subjects will also be provided with collection containers for storing the used pouches and a cooling bag for transportation of the used pouches to the study site.

From the day after *Visit 9*, subjects will completely switch from their own brand snus product to the chosen moist NP product for 14 days, using it *ad libitum*. Subjects will document their consumption once per day via an eDiary throughout the 14-day study period (recording the number of pouches used per day, the estimated usage time per pouch, and whether they opened a new can). Additionally, subjects will collect 4 used pouches on two separate days during the first week (Samples A) and on two separate days during the second week (Samples B), storing them in freezer (≤ -18°C). In total, 8 pouches will be collected per week.

At Visit 10 (14 days after *Visit 9*) the subjects will return to the study site in the morning. They will bring the collected frozen pouches for extraction analysis. During this visit, subjects will undergo interviews regarding any experienced AEs, the NP product used, and their compliance with the eDiary will be assessed. Additionally, a 24-hour urine collection (allowed time window: ±15 minutes) will begin in the morning. Subject will remain on-site until the following morning, using the selected NP product *ad libitum*. The urine will be continuously collected during this period for the analysis of BoE.

The subject may leave the study site after completion of the 24-hour urine collection and final assessment of AEs and concomitant medications. This is the end of the subject's study participation.

Investigational Products and dosage

Investigational product	Nicotine content per pouch/portion
NP 1 – moist nicotine pouch product (Part 2 and option in Part 3)	6 mg
NP 2 – moist nicotine pouch product (Part 2 and option in Part 3)	11 mg
NP 3 – moist nicotine pouch product (Part 2 and option in Part 3)	16.6 mg
Subject's own brand snus (comparator) (Part 1 and Part 2)	(optional)
Swedish pouch snus General G.3 Extra Strong Slim (comparator) (Part 2)	18 mg

Duration of IP use

Part 1: 14-days *ad libitum* use of the subject's own brand snus.
Part 2: single use (30 minutes) of 5 different products.
Part 3: 14-days *ad libitum* use of the selected NP.

Duration of each subject's involvement in the study

Each subject will participate in the study for a period of approximately 7 weeks, not including the preceding 5-week screening period.

Pharmacokinetic assessments – Part 2

Blood samples for analysis of PK parameters will be collected pre-use, and at 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h, and 6 h post-use. The PK parameters in the study will include $AUC_{0\text{-inf}}$, $AUC_{0\text{-last}}$, $AUC_{0\text{-1.5h}}$, C_{max}, T_{max}, and $T_{1/2}$.

Pharmacodynamic assessments – Part 2

The PD effects will be assessed by measuring pulse rate and subjective parameters (using VAS) from pre-use (15 minutes prior to IP use) to 6 hours post-use, at pre-defined time points. The subjective parameters that will be assessed are "craving", "satisfaction", "product-liking", and "intent to use again". In addition, "product-liking" *vs.* the subjects' usual oral tobacco/nicotine product of choice will be assessed using a 3-point scale questionnaire at 30 minutes post-use.

Analysis of biomarkers – Part 1 and Part 3

Analysis of urine concentrations of BoE (nicotine and its metabolites, NNN, NNAL, and anabasine) after 14 days of *ad libitum* use of an own brand snus product as well as after 14 days of exclusive, *ad libitum*, use of a moist NP product.

Nicotine extraction assessment – Part 2

Used pouches/portions will be collected after 30 minutes of use for the determination of residual nicotine in the IPs. The extracted amount (mg/unit) and extracted fraction (%) of nicotine will be assessed.

Extraction assessment – Part 1 and Part 3

For pouched products, the calculation of the extracted amount and fraction of nicotine, NNN, and NNK will involve subtracting the average of the pouches used by the subjects on two separate days during each 14-day usage period from the average of 10 unused pouches. For loose snus users, the calculation is based on a single unused reference portion. The weight of this reference portion is identical to the portion that each user prepares themselves during Part 2.

Safety assessments – Part 2 and Part 3

AEs will be collected through subject interviews and will also include any AEs reported spontaneously by the subjects, starting from the initiation of IP use (Visit 4) and continuing until the last IP use visit (Visit 10).

Statistical methods

The sample size has been calculated considering the primary endpoint: to show non-inferiority of the NP 11 mg product compared with the comparator product Swedish snus 18 mg.

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum, and maximum value, Q1-Q3 (interquartile range [IQR]). In addition, for applicable PK parameters, the geometric mean and coefficient of variation (CV) will be presented.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by IP, and by assessment time. Individual subject data will be listed by subject number, IP, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC, US).

For Part 2, baseline will be defined as the last data collection time point prior to each IP use. No adjustment for multiple comparisons will be performed. All formal comparisons will be made toward a designated reference product and all significant findings will be reviewed for relevance.

Generally, no imputation of data will be performed. In case of missing start and stop times of AEs that cannot be investigated further, missing data will be imputed according to a worst-case scenario, *i.e.*, start time will be imputed as the closest time point post intake of IP and end time as 23:59, resulting in the longest possible treatment emergent duration of the AE.

Spurious data will be evaluated continuously through data validation and, if connected to protocol deviations, evaluated case-by-case at the latest prior to declaration of clean file and database lock.

Deviations from the original statistical analysis plan (SAP) will be described in the clinical study report (CSR).

Methods for handling of missing, unused, and spurious data will be further specified in the SAP.

Study reporting

After completion of the study, an International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E3 guideline-compliant CSR will be prepared.

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3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Explanation
ADL	Activities of daily living
ADHD	Attention deficit hyperactivity disorder
AE	Adverse event
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma concentration *vs.* time curve
$AUC_{0\text{-inf}}$	AUC from 0 to infinity
$AUC_{0\text{-last}}$	AUC from 0 to time of last measurable plasma concentration
$AUC_{0\text{-1.5h}}$	AUC from time 0 to time 1.5 hours
BfR	German Federal Institute for Risk Assessment
BMI	Body mass index
BoE	Biomarkers of exposure
C_{max}	Maximum observed concentration
CRO	Contract research organization
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European Economic Area
E_{max}	Highest recorded change from baseline
$E_{max0\text{-}60}$	E_{max} from 0 to 60 minutes
ePRO	Electronic patient reported outcome
EU	European Union
FAS	Full analysis set
GCP	Good clinical practice
GDPR	General data protection regulation
HIV	Human immunodeficiency virus
HR	Heart rate
ICF	Informed consent form

Abbreviation	Explanation
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent ethics committee
IP	Investigational product
ISF	Investigator site file
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLOQ	Lower limit of quantification
MCQ	Multiple-choice question
MedDRA	Medical dictionary for regulatory activities
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N -nitrosonornicotine
NP	Nicotine pouch
NRT	Nicotine replacement therapies
OH-Cot	3'-trans-hydroxycotinine
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
PKAS	PK analysis set
PT	Preferred term
QC	Quality control
RAC	European Chemicals Agency´s Committee for Risk Assessment
RBM	Risk based monitoring
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Statistical analysis software
SD	Standard deviation
SDV	Source data verification
SIS	The Swedish Institute for Standards
SOC	System organ class
SOP	Standard operating procedures
$T_{1/2}$	Terminal elimination half-life
T_{Emax}	Time to reach E_{max}

Abbreviation	Explanation
$T_{Emax0-60}$	Time to reach $E_{max0-60}$
T_{max}	Time of occurrence of C_{max}
TMF	Trial master file
US	United States (of America)
VAS	Visual Analogue Scale
WHO	World Health Organization

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical emergencies contact

The Principal Investigator (PI) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes a serious adverse event (SAE) and is to be reported as such. Detailed SAE reporting procedures are described in Section 11.5.4.10.

In the case of a medical emergency, the Investigator may, during office hours, contact the Sponsor's medically responsible person (Table 4.1-1).

Table 4.1-1 Medical emergencies contact

Name	Function in the study	Contact information
[redacted]	[redacted]	[redacted]

5 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor

Swedish Match
Maria Skolgata 83
SE-118 53 Stockholm
Sweden

Sponsor's Medical Representative

[redacted]

Sponsor's Project Manager

[redacted]

Clinical conduct

Site 1:

Clinical Trial Consultants AB (CTC)
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SE-752 37 Uppsala, Sweden

Coordinating/Principal Investigator (Site 1)

[redacted]

Site 2:

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Ebbegatan 3
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Principal Investigator (Site 2)

[redacted]

Study management

Clinical Trial Consultants AB
Dag Hammarskjölds väg 10B
SE-752 37 Uppsala, Sweden

Clinical Research Manager

[redacted]

Biostatistician

[redacted]

Pharmacokineticist

[redacted]

Medical Writer

[redacted]

Laboratory (virology)	[redacted]
Laboratory (bioanalysis, urine biomarkers)	[redacted]
Laboratory (extraction)	[redacted]
Investigational product (IP) manufacturing	[redacted]
IP packaging and labelling	[redacted]
Electronic data capture (EDC) system provider	[redacted]

Signatures are provided in Section 19.

6 INTRODUCTION

6.1 Background

Tobacco harm reduction involves adopting strategies to minimize the health risks associated with tobacco use, particularly for individuals who cannot or do not wish to quit nicotine usage [1]. This approach includes transitioning from more harmful combustible cigarettes to potentially less harmful alternatives such as Swedish snus and nicotine pouches (NPs), providing viable options for nicotine delivery with potentially reduced health risks.

Use of oral tobacco is by definition unassociated with exposure to the many thousands of combustion compounds found in tobacco smoke (many of which are highly carcinogenic and may induce a state of systemic, chronic inflammation), or chronic irritation in the upper and lower airways resulting from the inhalation of tobacco smoke. Therefore, it is generally accepted that use of oral tobacco products has substantially lower health risks than cigarette smoking.

Nicotine is the substance that majorly contributes to the addictive properties of tobacco products. Nicotine exposure may contribute to adverse pregnancy outcomes. Additionally, oral tobacco products typically contain low levels of unwanted substances, including nitrosamines and polycyclic hydrocarbons, which have been classified as human carcinogens. While the health effects of oral tobacco are substantially smaller compared to cigarette smoking, some adverse effects cannot be ruled out, particularly those related to nicotine exposure.

Epidemiological data, based on the historical use of traditional Swedish snus in Sweden, shows a highly reduced risk profile for Swedish snus compared to cigarettes. Due to the product category's relative novelty, long-term epidemiological data on NPs is not yet at hand. Given the reduced levels of toxicants in NPs compared to traditional Swedish snus, the epidemiological data on snus can be extrapolated to assess the long-term health risks associated with NP use. The extrapolation is justified as long as the nicotine exposure from NPs does not exceed that from traditional Swedish snus. With a daily consumption of 32.1 g loose snus [2], an extraction of 24 % after one hour of use, and an average nicotine concentration of 0.8 – 1.0%, the daily exposure of nicotine is estimated to be between 62 – 77 mg. NPs, however, have different extraction rates. In this calculation, data from marketed NPs was used. An extraction rate of 39% after one hour of use was estimated, and the average consumption of pouches was found to be between 8.6 – 12.3 per day [3]. A hypothetical nicotine amount of 16.6 mg per pouch would then result in a nicotine exposure of 56 – 80 mg, which is similar to the epidemiologic data for loose snus.

Traditionally, Swedish snus pouch products have contained around 8-10 mg/g of nicotine [4], with the market offering various products with both lower and substantially higher nicotine levels (up to 45 mg/unit). Before the first pouched snus, SMOKELESS, weighed 1 gram, was introduced in 1973, there was only loose snus. More of the famous brands followed with portion-packed versions, including Tre Ankare in 1976, General® in 1985, and Göteborgs Rapé in 1997. Between 1986-2000, the use of loose snus went from being used by 95% to 58% of exclusive snus users.

While most NP products on the Swedish market contain between 1.5 mg and 17 mg of nicotine per pouch, there are concerns about products containing significantly higher amounts. In extreme cases, certain NPs can contain up to 150 mg of nicotine per pouch. This high amount poses serious health risks, especially for new users or those with low nicotine tolerance. Due to these risks and other factors (see below), nicotine limits for NPs have been under discussion. The Swedish Institute for Standards (SIS) set a 20 mg/unit limit in 2020

(SIS/TS 72:2020) and updated this standard in 2024 (SIS/TS 72:2024), maintaining the same limit. The German Federal Institute for Risk Assessment (BfR) recommended a limit of 16.6 mg/unit in their 2022 updated opinion (BfR Opinion no. 023/2022, 7 October 2022) [5].

Long- and short-term health effects of NPs are debated based on three key factors:

- Acute toxicity: The European Chemicals Agency´s Committee for Risk Assessment (RAC) classifies nicotine as Acute Tox. 2 (oral) with the hazard warning 'H300: Fatal if swallowed' and an acute toxicity estimate of 5 mg/kg bodyweight. This classification is now enforced by EU Regulation 2018/1480.
- Addiction potential: Nicotine addiction potential is typically assessed through pharmacokinetic/pharmacodynamic (PK/PD) studies, where higher maximum observed plasma concentration (C_{max}) and shorter time to maximum (T_{max}) indicate greater addiction potential. Balancing this, products must achieve a sufficiently high C_{max} and short enough T_{max} to serve as viable alternatives for users of combustible cigarettes.
- Long-term health effects: Beyond addiction, concerns revolve around nicotine´s long-term health impacts. Nicotine is associated with cardiovascular effects like temporary increases in heart rate (HR) and blood pressure [6, 7]. However, epidemiolocal studies have not conclusively linked snus use to cardiovascular disease onset, suggesting historical nicotine exposure levels among snus users may not significantly elevate the risk of developing cardiovascular disease.

6.2 Study rationale

To examine the impact of nicotine content in NPs on nicotine exposure, it is essential to assess both addiction potential through PK/PD studies and evaluate real-life nicotine exposure using biomarkers of exposure (BoE). The nicotine content in tobacco-based snus products varies substantially, and it is important to ensure that NPs on the market do not have a higher addiction potential than these products; hence the inclusion of a tobacco-based snus product (18 mg/pouch) representing the higher end of marketed nicotine contents for comparisons in Part 2 of the study.

Given the extensive research on Swedish snus, the nicotine exposure from Swedish snus provides a benchmark for assessing the long-term health effects of NPs. Therefore, the results from this study, given that the nicotine exposure does not exceed that of Swedish snus, can later be used to evaluate the long-term health effects of NPs.

Nicotine exposure is determined by a combination of product-specific factors and usage patterns. User patterns are likely to change when switching from a usual brand snus product to a NP product. In particular, if the change in product would have caused a lower or higher nicotine exposure with an unchanged usage pattern, it is conceivable that the user would adjust their usage pattern to maintain their previous level of nicotine exposure. The extent to which such self-titration occurs is not well described for snus and unknown for NPs, although self-titration is known to occur with combustible cigarettes.

The overall aim of the study is to achieve a comprehensive understanding of how nicotine content affects actual exposure, addiction potential, and whether the long-term health risks of NPs can be estimated based on the epidemiological studies available for tobacco-based snus. Specifically:

- Part 1 aims to provide a baseline assessment in order to evaluate exposure in users of tobacco-based snus, which will involve measuring product usage and exposure to nicotine.

- Part 2 aims to evaluate addiction potential of NPs in comparison to tobacco-based snus (own brand and a snus product representing the higher end of marketed nicotine contents) through a PK/PD study and assess product-specific nicotine exposure under controlled use.
- Part 3 aims to provide insight into nicotine self-titration behaviors, considering both the choice of product strength and subsequent *ad libitum* usage patterns compared to Part 1, and linking these to the product-specific factors measured in Part 2.
- Additionally, N -nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in used pouches/portions will be analyzed. Urine levels of BoE (nicotine, its metabolites, NNN, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and anabasine) will also be analyzed. Both of these analyses will be performed and compared for Part 1 and Part 3.

6.3 Risk/benefit assessment

6.3.1 Risk assessment

All research subjects must be daily Swedish snus users for at least 1 year, consuming at least 5 pouches/portions of snus per day. They must be willing and able to use both Swedish snus and NPs with high nicotine content. Consequently, the subjects are well acquainted with and accustomed to the effects of nicotine, minimizing the risk of developing any new nicotine dependency. Part 1 of the study will involve *ad libitum* usage of the subjects´ own brand snus product. The subjects will follow their regular pattern of use for 14 days, documenting their consumption. As the subjects will be using their snus product as they normally would, this part of the study does not pose any additional risks or change the nicotine exposure.

Part 2 of the study will involve single-dose administrations of one Swedish snus product (General G.3 Extra Strong Slim 18 mg), three NP products (6 mg, 11 mg, and 16.6 mg), and the subjects´ own brand snus product. Although the nicotine content of one or more of these products may be higher than some subjects´ usual brand, subjects must abstain from tobacco/nicotine products for at least 12 hours before use and will only be administered one product during study visits. Therefore, it is reasonable to anticipate that the daily nicotine exposure will be lower during these visits.

Before starting Part 3 of the study, subjects will receive one can of each NP product to test at home over 2-3 days. This is to ensure they find a suitable replacement for their own brand snus product, which they will use *ad libitum* for 14 days. During this period, subjects can adjust their usage patterns, using the products as much or as little as they wish. One aim of the study is to investigate the extent of adjustment among snus users who switch to NPs. Because there is insufficient information about the degree of adjustment, it cannot be determined with certainty that the nicotine exposure will match the subjects´ normal levels. If the adjustment is incomplete, nicotine exposure could be higher or lower than usual. However, consumers are regularly exposed to snus and NP products with higher nicotine content than those in this study. Additionally, the NP products in this study are below the limits set by SIS and BfR, indicating that the risks are expected to be low even if some subjects may have a higher nicotine exposure than their usual.

Subjects who intend to change their nicotine consumption habit or stop using nicotine-containing products, and/or are pregnant, breastfeeding, or intend to become pregnant during the study, and/or subjects with a history or presence of diagnosed hypertension or cardiovascular disease or other medical condition who may be particularly vulnerable to nicotine exposure, are excluded from participation.

The nicotine in the NP products is of pharmaceutical grade, same as the nicotine used in nicotine replacement (NRT) products (*e.g.*, gum, lozenges, mouth spray *etc*.). Aside from nicotine, all ingredients used in the NP products are approved for use in food.

The potential adverse effects of the study procedures are expected to be minor and clinically insignificant, based on experience from clinical studies on similar products [8-15]. Notably, previous clinical studies with similar products have reported no adverse events (AEs) other than those likely attributed to nicotine exposure, such as salivation, nausea, and dyspepsia.

The PIs at the study sites will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the study. The medical staff at the study sites have extensive experience in clinical studies, and there are adequate procedures in place to handle unexpected and expected adverse reactions in the study subjects.

Aside from the risks related to the IPs, as detailed above, there may also be risks related to the medical devices used in the study during Part 2 (*e.g.*, indwelling venous catheters). However, these devices are used in routine medical care and the risk associated with their use is considered low and ethically justifiable. The repeated blood sampling for PK analysis will be conducted during a 2-week period to give sufficient time between each blood sample occasion. Study specific evaluations and sampling procedures, such as blood pressure measurements using a blood pressure cuff and frequent blood sampling, may cause transient discomfort but the risk is deemed to be low and ethically justifiable. Overall, the combined safety data from previous pre-clinical and clinical studies have not revealed any safety issues that would outweigh the expected benefits of the study.

6.3.2 Benefit assessment

In analogy with a regular phase I study in healthy volunteers, there is no direct benefit for the subjects to participate in the study, aside from a brief medical examination, which may provide them with information on their general state of health. However, the participation of the subjects is important to estimate the long-term health risks of NPs, which can benefit all consumers of these products.

6.3.3 Risk/benefit conclusion

The long-term health risks of NPs are not known. Since they contain fewer harmful substances than tobacco-based snus, as they do not contain tobacco, the extensive research available for tobacco-based snus can be used to estimate the long-term health risks of NPs, provided that the nicotine exposure from NPs is not higher than from tobacco-based snus.

Nicotine exposure is primarily dependent on the nicotine content and consumers´ usage patterns. The nicotine content in tobacco-based snus and NPs varies greatly and differs between markets (1.5-150 mg per pouch). In Sweden, SIS has established a limit of 20 mg per pouch. NPs with a nicotine content below this threshold are deemed safe for consumer use.

The overarching aim of the study is to achieve a comprehensive understanding of how nicotine content affects actual nicotine exposure, addiction potential, and whether the long-term health risks of NPs can be estimated based on the epidemiological studies available for tobacco-based snus. As the use of NPs has increased significantly in recent years and the products vary greatly in nicotine content, it is important to try to estimate how this affects, for example, long-term cardiovascular health.

In this study, NPs with 3 different nicotine contents will be used, all of which are within the recommended limits and reflect the products commonly used by consumers in Sweden. The

results of the study can guide the establishment of recommendations and regulations regarding appropriate nicotine limits to restrict the nicotine content of NPs.

In Part 3 of the study, there is a potential risk that some subjects may be exposed to higher levels of nicotine than usual during the 14-day period. However, the nicotine contents in the NP products are below the limits set by SIS and BfR, indicating that the associated risks are expected to be low. Additionally, many consumers already use snus or NP products with high nicotine content, and the study products are similar to those available on the market. Therefore, while there is a risk of increased nicotine exposure, the overall benefit of the study is supported by the low expected risk and the relevance of the study products to existing consumer habits.

The potential AEs and risks associated with the study procedures are likely to be minor and/or clinically insignificant. It is concluded that the planned study assessments suffice to meet the scientific and medical goals. Therefore, the potential benefits of the study outweigh the potential risks for the daily habitual tobacco-based snus users that will participate in this study.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objectives and endpoints

The study objectives and endpoints are presented in Table 7.1-1 (primary), Table 7.2-1 (secondary), and Table 7.3-1 (exploratory).

Table 7.1-1 Primary study objective and endpoint

Primary objective	Endpoint	Assessments	Analysis
To demonstrate that the moist nicotine pouch (NP) 11 mg product does not result in substantially higher nicotine exposure compared to the comparator product Swedish snus 18 mg.	To compare nicotine exposure, as measured by baseline-adjusted area under the plasma concentration *vs.* time curve from 0 to infinity ($AUC_{0\text{-}inf}$) based on nicotine plasma concentrations, between the moist NP 11 mg product and the comparator product, Swedish snus 18 mg. (The goal is to demonstrate that the upper bound of the 95% confidence interval for the ratio for nicotine exposure between the moist NP 11 mg product and the comparator product is at or below 1.25.)	(Section 11.4.1)	See Section 17.6.1.

7.2 Secondary objectives and endpoints

The secondary study objectives and endpoints are presented in Table 7.2-1.

Table 7.2-1 Secondary study objective and endpoint

No.	Secondary objectives	No.	Endpoints	Assessments	Analysis
1.	To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	1.	The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	(Section 11.5.2)	See Section 17.7.1.
2.	To compare the pharmacokinetic (PK) profile between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	2a.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, in the non-adjusted and baseline-adjusted PK parameters based on plasma concentrations of nicotine: $AUC_{0\text{-}inf}$, maximum observed plasma concentration (C_{max}), time to C_{max} (T_{max}), AUC from 0 to 1.5 hours ($AUC_{0\text{-}1.5h}$), AUC from 0 to time of last measurable time point ($AUC_{0\text{-}last}$), and terminal elimination half-life ($T_{1/2}$).	(Section 11.5.1)	See Section 17.7.2.
		2b.	To compare nicotine exposure, as measured by non-adjusted and baseline-adjusted $AUC_{0\text{-}inf}$, between the moist NP 6 mg and NP 16.6 mg products and the	(Section 11.5.1)	See Section 17.7.2.

			comparator products, Swedish snus 18 mg and own brand snus product. This comparison also includes the NP 11 mg product and the own brand snus product. (The goal is to demonstrate that the upper bound of the 95% confidence interval for the ratio for nicotine exposure between the moist NP products and the comparator products is at or below 1.25.)		
3.	To assess the pharmacodynamic (PD) effects, measured as pulse rate and subjective outcome measures, of the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	3a.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, for the highest recorded increase (E_{max}), the time to E_{max} (T_{Emax}), the E_{max} from time 0 to 60 min ($E_{max0-60}$), and the time to reach $E_{max0-60}$ ($T_{Emax0-60}$) in pulse rate from baseline, measured using a pulse oximeter after IP use.	(Section 11.5.3.1)	See Section 17.7.3.
		3b.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, for the largest recorded decrease (E_{max}) in the subjective parameter "craving" and the highest recorded value for "satisfaction", measured using a 100 mm visual analog scale (VAS) after IP use.	(Section 11.5.3.2)	See Section 17.7.3.
		3c.	The difference between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product, for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 30 minutes after IP use.	(Section 11.5.3.2)	See Section 17.7.3.
4.	To evaluate the safety and tolerability of the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	4.	Frequency, intensity, and seriousness of adverse events (AEs).	(Section 11.5.4)	See Section 17.7.4.

7.3 Exploratory objectives and endpoints

The exploratory study objectives and endpoints are presented in Table 7.3-1.

Table 7.3-1 Exploratory objectives and endpoints

No.	Exploratory objectives	No.	Endpoints[1]	Assessments	Analysis
1.	To evaluate the impact on "product-liking" *vs.* the subjects' usual Swedish snus product of choice by administering single doses of the moist NP 6 mg, 11 mg, and 16.6 mg	1.	Difference in "product-liking" *vs.* the subjects' usual Swedish snus product of choice between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator product, Swedish snus 18 mg, measured on a 3-point scale using	(Section 11.6.1.1)	See Section 17.8.1.

	products and the comparator product, Swedish snus 18 mg.		a multiple-choice question (MCQ) 30 minutes after IP use.		
2.	To compare the nicotine extraction normalized PK parameters $AUC_{0\text{-}inf}$ and C_{max} between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	2.	Difference in nicotine extraction normalized $AUC_{0\text{-}inf}$ and C_{max} between the moist NP 6 mg, 11 mg, and 16.6 mg products and the comparator products, Swedish snus 18 mg and own brand snus product.	(Section 11.6.1.2)	See Section 17.8.2.
3.	To analyze the pattern of use during each of the two 14-day *ad libitum* usage periods of either own brand snus product usage or complete substitution with a moist NP product.	3.	Difference in the pattern of use during each of the two 14-day *ad libitum* usage periods of either own brand snus product usage or complete substitution with a moist NP product.	(Section 11.6.1.3)	See Section 17.8.3.
4.	To compare the PK profile between the selected strength NP product and the usual brand snus product.	4.	To compare nicotine exposure (in Part 2), as measured by baseline-adjusted $AUC_{0\text{-}inf}$ and C_{max}, between the selected strength NP product (in Part 3) and the usual brand snus product (in Part 1).	(Section 11.4.1)	See Section 17.8.4.
5.	To compare urine concentrations and amounts of BoE (nicotine and its metabolites, NNN, NNAL, and anabasine) before and after complete substitution of own brand snus product with a moist NP product for 14 days.	5.	Difference in creatinine-normalized urine concentrations and amounts of BoE (nicotine and its metabolites, NNN, NNAL, and anabasine) before and after complete substitution of own brand snus product with a moist NP product for 14 days.	(Section 11.6.1.5)	See Section 17.8.5.
6.	To compare the extracted amounts and fractions of nicotine, NNN, and NNK from used and collected own brand snus pouches/portions and used moist NPs.	6.	Difference in the extracted amounts (mg/unit) and fractions (%) of nicotine, NNN, and NNK from used and collected pouches/portions during each of the two 14-day *ad libitum* usage periods of either own brand snus product usage or complete substitution with a moist NP product.	(Section 11.6.1.2)	See Section 17.8.6.
7.	To correlate the extracted amounts of nicotine, NNN, and NNK, multiplied by the number of pouches/portions used per day on average, with urine concentrations of BoE.	7.	The correlation between the extracted amounts (mg/unit) of nicotine, NNN, and NNK, multiplied by the number of pouches/portions used per day on average, and the creatinine-normalized urine concentrations of BoE before and after complete substitution of the own brand snus product with a moist NP product for 14 days.	(Section 11.6.1.2 and 11.6.1.5)	See Section 17.8.7.

1. Loose snus users will be analyzed as a subgroup for all exploratory endpoints.

8 STUDY DESIGN

8.1 Overall study design and schedule of events

This is a multi-center, open-label, three-part study:

- *Part 1:* a multi-center, non-randomized observational part involving use of the subject´s own brand Swedish snus product of choice (hereafter, called own brand snus) *ad libitum* (see Section 8.1.1).
- *Part 2*: an open-label, multi-center, randomized, 5-way cross-over, single use part. The investigational products (IPs) include three moist NP products, containing 6 mg/unit, 11 mg/unit, and 16.6 mg/unit of nicotine. The comparator products include one Swedish snus 18 mg/unit product and subject´s own brand snus product (see Section 8.1.2).
- *Part 3:* a multi-center, non-randomized interventional part involving complete substitution of own brand snus product with a NP product for *ad libitum* use (see Section 8.1.3).

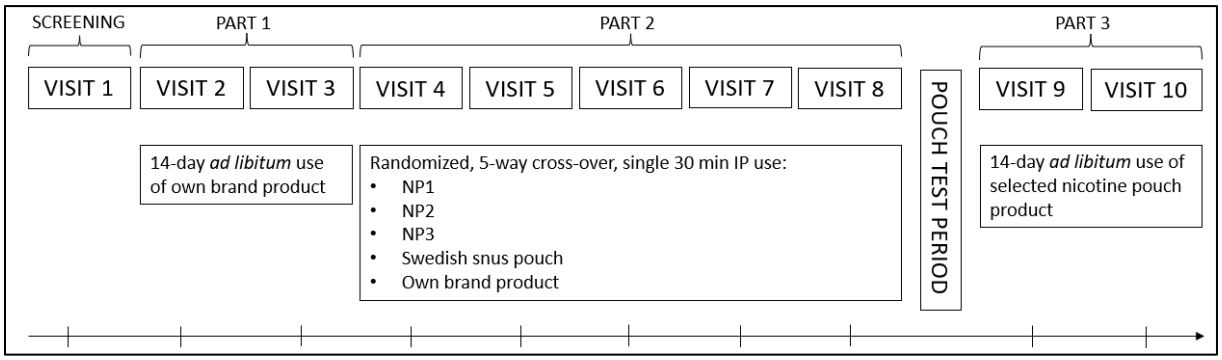
The study will include approximately 53 subjects with the aim of randomizing at least 53 subjects in Part 2 and achieving 45 evaluable subjects (for Part 2).

The subjects will be healthy male and female subjects who have used Swedish snus products for ≥1 year, with a minimum daily consumption of 5 pouches/portions. Efforts will be made to include at least 21 female subjects (approximately 40%) in Part 2. However, a minimum of 11 female subjects (approximately 20%) will be considered acceptable. Additionally, efforts will be made to include 10 to 15 users of loose snus.

Each subject will participate in the study for approximately 7 weeks, not including the preceding up to 5-week screening period.

An overview of the study design is shown in Figure 8.1-1. An overall schedule of event is presented in Table 8.1-1 and a detailed schedule of events for each IP use visit in Part 2 is presented in Table 8.1-2. Study assessments are described in Section 11.

Figure 8.1-1 Overview of the study design



The screening visit (*Visit 1*) will take place within 5 weeks prior to start of Part 2 (Visit 4) and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, collection of medical history, a brief physical examination, serology tests, electrocardiogram (ECG), vital signs (pulse rate and blood pressure), height, weight, and body mass index (BMI) assessments. During the screening visit, subjects will complete a nicotine dependence questionnaire. They will also select the Swedish snus product, either a pouch or loose snus, which they will use exclusively during Part 1 of the study. Subjects will purchase

this product themselves. The chosen product should be the brand they predominantly used in the past month unless they did not exclusively use a single brand. This selection will determine the nicotine strength and flavor variation, as these factors vary within the same brand. For Part 2 of the study and for chemical analyses, the Sponsor will purchase the selected snus product. For details, refer to Table 8.1-1.

8.1.1 Part 1: Ad libitum use of own brand snus

At Visit 2 (first day of Part 1), eligible subjects will return to the study site to receive collection materials for used pouches and undergo eDiary training and interviews regarding their health status and medications used.

After Visit 2, subjects will exclusively use their product of choice *ad libitum*, following their regular pattern of use. They will document their consumption via an eDiary (ViedocMe) during a 14-day study period, recording the number of pouches/portions used per day, the estimated usage time per pouch/portion, and whether they opened a new snus can for pouch/portion usage once per day. Also, subjects will collect 4 used pouches/portions on two separate days the first week (Samples A) and on two separate days the second week (Samples B) and store these in a freezer (≤ -18°C). In total, 8 pouches/portions will be collected per week. Subjects will receive instructions on how to collect used pouches/portions and will be provided with collection containers for storing them and a cooling bag for transportation of the used pouches to the study site.

At Visit 3 (14 days [allowed time window: +3 days] after Visit 2) the subjects will return to the study site in the morning. They will bring the collected frozen pouches/portions for extraction analysis. During this visit, subjects will undergo interviews regarding their health status and medications used, the specific Swedish snus product they used (including its strength and flavor), and their compliance with the eDiary will be assessed. Additionally, a 24-hour urine collection (allowed time window: ±15 minutes) will begin in the morning. Subject will remain on-site until the following morning, using the snus product *ad libitum*. The urine will be continuously collected during this period for the analysis of BoE.

8.1.2 Part 2: single IP use in a 5-way cross-over

At Visit 4, the subjects will return to the study site for the first IP use visit of Part 2.

Subjects shall abstain from tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for 12 hours prior to each IP use visit (Visits 4 to 8). All IP use sessions will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

The IPs will be administered as single pouches/portions in a pre-determined randomized order. Randomization will take place before the first IP use of Part 2.

Subjects will keep the pouch/portion between their lip and gum for 30 minutes and will be instructed not to eat, drink, chew gum, or brush their teeth for 30 minutes before, during, and 30 minutes after the IP use.

After 30 minutes, each used pouch/portion will be collected and frozen (-20°C) pending analysis of residual nicotine content. Unused pouches/portions from the same batch will serve as references and will be stored at -20°C pending analyses.

Blood samples for the assessment of nicotine plasma levels and PK parameters will be collected at pre-defined time points from pre-use to 6 hours after each IP use. The PD effects of the IPs will be determined using pulse rate measurements and subjective parameters (using VAS questions) at the same pre-defined time points as well as a multiple-choice question (MCQ) 30 minutes after IP use.

AEs will be collected through subject interviews and will also include any AEs reported spontaneously by the subjects, starting from the initiation of IP use (Visit 4), and continuing until the last IP use visit (Visit 8).

Visits 5 to 8 will follow the same schedule as Visit 4. Each visit will be scheduled on separate days, preferably with at least 24 hours between visits. However, it is acceptable to have visits on consecutive days, as long as all 5 visits are not scheduled consecutively.

At the end of *Visit 8*, the subject will receive one can of each of the three moist NP products with varying strengths (6 mg, 11 mg, and 16.6 mg) used in Part 2. Importantly, the subjects will be blinded to the nicotine strength of these three products. The subject will test these at home over 2-3 days to ensure they find a suitable replacement for their own brand snus product to be used in Part 3.

8.1.3 Part 3: Ad libitum use of nicotine pouch product

At Visit 9 (first day of Part 3), the subjects will return to the study site and then the chosen NP product (6 mg/unit, 11 mg/unit or 16.6 mg/unit) will be documented. Based on the subject's reported consumption, they will be provided with the NP product to be exclusively used *ad libitum* for 14 days (allowed time window: +3 days). During this visit, subjects will undergo interviews regarding any experienced AEs. Subjects will be reminded on how to document their consumption using the eDiary and how to collect used pouches. The subjects will also be provided with collection containers for storing the used pouches and a cooling bag for transportation of the used pouches to the study site.

From the day after *Visit 9*, subjects will completely switch from their own brand snus product to the chosen moist NP product for 14 days, using it *ad libitum*. Subjects will document their consumption once per day via an eDiary throughout the 14-day study period (recording the number of pouches used per day, the estimated usage time per pouch, and whether they opened a new can). Additionally, subjects will collect 4 used pouches on two separate days during the first week (Samples A) and on two separate days during the second week (Samples B), storing them in freezer (≤ -18°C). In total, 8 pouches will be collected per week.

At Visit 10 (14 days after *Visit 9*) the subjects will return to the study site in the morning. They will bring the collected frozen pouches for extraction analysis. During this visit, subjects will undergo interviews regarding any experienced AEs, the NP product used, and their compliance with the eDiary will be assessed. Additionally, a 24-hour urine collection (allowed time window: ±15 minutes) will begin in the morning. Subject will remain on-site until the following morning, using the selected NP product *ad libitum*. The urine will be continuously collected during this period for the analysis of BoE.

The subject may leave the study site after completion of the 24-hour urine collection and final assessment of AEs and concomitant medications. This is the end of the subject's study participation.

Table 8.1-1 Overall schedule of events

Events	CSP section	Visit 1 Screening	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Test period	Visit 9	Visit 10
			Part 1		Part 2						Part 3	
Informed consent	11.3.1	X										
Demographics	11.3.2	X										
Medical/surgical history	11.3.3	X										
History of and current nicotine product use	11.3.4	X										
Nicotine dependency test	11.3.16	X										
Document choice of own brand product	11.3.4	X										
Inclusion/exclusion criteria	9.4/9.5	X			X[1]							
Weight, height, and BMI	11.3.6	X										
Physical examination	11.3.7	X										
Vital signs (blood pressure and pulse rate)	11.3.8	X										
Electrocardiogram	11.3.9	X										
HIV, Hepatitis B and C	11.3.10	X										
Pregnancy test[2]	11.3.11	X		X	X	X[3]	X[3]	X[3]	X[3]		X[3]	X
Urine drug screen[4]	11.3.12	X		X	X							
Alcohol screen[4]	11.3.13	X		X	X							
eDiary training	11.2		X								(X)	
eDiary[5] (*ab lib* use periods)	11.2		------X-------								------X-------	
eDiary compliance check	11.2			X								X
Hand-out: used pouches collection material			X									
Use of own brand product			------X-------									
Used pouch/portion sampling[6] (by subjects)			------X-------									
Used pouch/portion collection				X								
Urine collection (24-hour, in-clinic)				X								X

Randomization	9.9				X							
IP use	10.5				X[7]	X[7]	X[7]	X[7]	X[7]			
PK blood sampling (plasma)	11.4.1				X[7]	X[7]	X[7]	X[7]	X[7]			
Subjective effects (VAS questions and MCQ)	11.5.3.2 11.6.1.1				X[7]	X[7]	X[7]	X[7]	X[7]			
Pulse rate (pulse oximeter)	11.5.3.1				X[7]	X[7]	X[7]	X[7]	X[7]			
IP collection	11.5.2				X[7]	X[7]	X[7]	X[7]	X[7]			
Hand-out: IP for test period									X			
Usage of test IP										X		
Handout: selected IP											X	
Usage of selected IP											------X-------	
Used pouch sampling[6] (by subjects)											------X-------	
Used pouch collection												X
Return of unused IP												X
Baseline symptoms	11.3.14	X	X	X								
AEs	11.5.4				X	X	X	X	X		X	X
Prior and concomitant medications	11.3.15	X	X	X	X	X	X	X	X		X	X

AE=adverse event, BMI= body mass index, HIV=human immunodeficiency virus, IP=investigational product, MCQ=multiple-choice question, VAS=visual analogue scale.

1. Confirmation of eligibility criteria.
2. Only subjects of childbearing potential.
3. Only at the discretion of the Investigator on Visits 5-9.
4. Additional drug and alcohol tests may be performed at the discretion of the Investigator during IP use visits.
5. eDiary to be filled in once daily.
6. Twice per week (4 pouches/portions on two separate days per week will be collected: in total 8 pouches/portions per week). Collected pouches/portions will be brought to the site at the next study visit.
7. The detailed timing of assessments is outlined in Table 8.1-2.

Table 8.1-2 Part 2: Detailed schedule of events for each IP use visit (Visits 4-8)

Visits 4-8																
Assessment / Time point	**Admission**	**-00:15**	**-00:10**	**-00:01**	**00:00**	**00:05**	**00:10**	**00:15**	**00:20**	**00:30**	**00:40**	**01:00**	**01:30**	**02:00**	**04:00**	**06:00**
Inclusion/exclusion criteria	X[1]															
Urine drug screen	X[2]															
Alcohol screen	X[2]															
Pregnancy test	X[3]															
Randomization	X[4]															
IP use					X											
IP collection										X						
PK blood sampling (plasma)			X[5]			X	X	X	X	X	X	X	X	X	X	X
VAS question ("craving")			X			X	X	X	X	X	X	X	X	X	X	X
VAS question ("satisfaction")						X	X	X	X	X	X	X	X	X	X	X
VAS ("product-liking" and "intent to use again")										X						
MCQ ("product-liking" *vs.* usual product)										X						
Pulse rate (pulse oximeter)		X				X	X	X	X	X	X	X	X	X	X	X
Handout of IP for test period																X
Baseline symptoms	X[6]															
AEs	X[7]															
Prior and concomitant medications	X															

1. Only at Visit 4. Confirmation of eligibility before randomization.
2. Only at Visit 4. Additional drug and alcohol tests may be performed during IP use visits at the discretion of the Investigator.
3. Only subjects of childbearing potential. Additional tests at the discretion of the Investigator on Visits 5-8.
4. Randomization occurs only on Visit 4.
5. Pre-use PK-sample taken at any time within 10 min to -1 min prior to IP use, except in direct association with the pre-use pulse rate assessment at -15 min.
6. Only on Visit 4. Baseline symptoms experienced prior to first IP use.
7. AEs experienced from first IP use.

8.2 Rationale for study design

This is a multi-center, open-label, study in three parts:

- *Part 1:* a multi-center, non-randomized observational part involving use of the subject´s own brand Swedish snus product of choice (hereafter, called own brand snus) *ad libitum*.
- *Part 2*: an open-label, multi-center, randomized, 5-way cross-over, single dose use part. The IPs include three moist NP products, containing 6 mg/unit, 11 mg/unit, and 16.6 mg/unit of nicotine. The comparator products include one Swedish snus 18 mg/unit product and subject´s own brand snus product.
- *Part 3:* a multi-center, non-randomized interventional part involving complete substitution of own brand snus product with a NP product for *ad libitum* use.

All study subjects will perform all three study parts in a consecutive order.

In Part 2, a cross-over design was chosen to yield a more efficient evaluation of NPs than a parallel study design, *i.e*., fewer subjects are required since each subject will serve as its own control. To avoid carry-over effects, subjects will abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for at least 12 hours prior to each IP use visit (Visits 4-8).

In Part 2, randomization will be used to minimize bias in the assignment of subjects to an IP use sequence and to increase the likelihood that known and unknown subject attributes (*e.g*., demographic and baseline characteristics) are evenly balanced.

In Part 1, product usage and exposure to nicotine, NNN, and NNK, as well as urine concentrations of nicotine and its metabolites, NNN, NNAL, and anabasine will be assessed during *ad libitum* usage of the subjects´ own brand snus product. These measurements will serve as baseline measures, with each subject acting as their own reference. In Part 3, this will be repeated, but the subjects will use an NP product instead. These two *ad libitum* periods will provide insights into self-titration behaviors and, link them to the product-specific factors measured in Part 2.

9 STUDY POPULATION

Prospective approval of deviations from the eligibility criteria, also known as protocol waivers or exemptions, is not permitted.

9.1 Recruitment

Subjects will be recruited from CTC's database of healthy volunteers, as well as from strategic marketing campaigns. Advertisements in social media and other media (newspapers, internet, radio, local distribution of flyers *etc*.) will be used to reach the target audience. The advertisement texts approved by the independent ethics committee (IEC) will be used to create all materials (digital, radio and/or print) for recruitment.

9.2 Screening and enrollment log

Investigators must keep a record of all screened subjects even if they were not subsequently included in the study. This information is necessary to verify that subjects were selected without bias. The reason for screening failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects that were included but did not complete the study.

A screening number generated automatically in the electronic case report form (eCRF) will be allocated to each subject in connection with the informed consent process at the screening visit (Visit 1). The screening number will allow identification of subjects irrespective of their possible eligibility for the study.

Eligible subjects will be assigned a 3-digit randomization number prior to the first IP use. If a subject is unable to receive the planned initial IP use within 35 days after screening (*i.e.*, the time interval between signing informed consent and the first IP use in Part 2) the subject should undergo re-screening before continuing in the study.

9.3 Number of subjects

The study will include approximately 53 subjects with the aim of randomizing at least 53 subjects in Part 2 and achieving 45 evaluable subjects (for Part 2).

Efforts will be made to include at least 21 female subjects (approximately 40%). However, a minimum of 11 female subjects (approximately 20%) will be considered acceptable.

Additionally, efforts will be made to include 10 to 15 regular users of loose snus. These users will use a loose snus of their choice as their own brand product during Part 1 and 2.

For the replacement of subjects who discontinue the study, see Section 9.8.3.

9.4 Inclusion criteria

For inclusion in the study, the subjects must fulfill the following criteria:

1. Willing and able to give written informed consent for participation in the study.
2. Subjects who have used Swedish snus products for ≥1 year, with a minimum daily consumption of 5 pouches/portions, who are willing and able to use both Swedish pouch snus and NPs with high nicotine content while abstaining from other tobacco/nicotine products during the study. (Dual use of Swedish snus and NPs before inclusion will be permitted, but subjects should exclusively use their usual brand of a Swedish snus product [including strength and flavor] *ad libitum* during Part 1.)
3. Healthy male or female subject aged 19 to 60 years, inclusive.

4. Medically healthy subject without abnormal clinically significant medical history, physical findings, vital signs, ECG, and hepatitis B/C and human immunodeficiency virus (HIV) results at the time of the screening visit, as judged by the Investigator.
5. **Female subjects of childbearing potential** must practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) or must agree to use a highly effective method of contraception with a failure rate of <1 % to prevent pregnancy for the duration of the study.

   The following are considered highly effective methods of contraception:

   - combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal),
   - progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable),
   - intrauterine device or intrauterine hormone-releasing system.

9.5 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. A history of diagnosed hypertension or any cardiovascular disease, or ongoing manifestations of hypertension or any cardiovascular disease as judged by the Investigator.
2. Any surgical or medical condition, including abnormal salivation (also pharmaceutically induced), or history thereof, which, in the judgment of the Investigator, might interfere with the absorption, distribution, metabolism or excretion of the IP or may either put the subject at risk because of participation in the study, influence the results, or the subject's ability to participate in the study.
3. A history of diagnosed severe allergy/hypersensitivity or ongoing manifestations of severe allergy/hypersensitivity to aroma compounds (including fragrances and/or flavorings), as judged by the Investigator.
4. Subjects with poor venous access or being scared of needles.
5. Any planned major surgery within the duration of the study.
6. Subjects who are pregnant, currently breastfeeding, or intend to become pregnant during the course of the study.
7. Any positive result at the screening visit for serum hepatitis B surface antigen, hepatitis B and C antibodies, and/or HIV.
8. Positive screening result for drugs of abuse or alcohol at the screening visit or on admission to the study site prior to IP use. Positive results that are expected given the subject's medical history and prescribed medications can be disregarded as judged by the Investigator.
9. History of alcohol abuse or excessive intake of alcohol, as judged by the Investigator.
10. Presence or history of drug abuse, as judged by the Investigator.
11. History of, or current use of anabolic steroids, as judged by the Investigator.
12. Current, ongoing use of beta-adrenergic blocking agents (beta blockers) or attention deficit hyperactivity disorder (ADHD) medications, including *pro re nata* (as needed) use.

13. Plasma donation within 1 month of screening or blood donation (or corresponding blood loss) during the last 3 months prior to screening.
14. Subjects who intend to change their nicotine consumption habit, including the intention to stop using nicotine products, within the next 3 months of the screening visit, as judged by the Investigator.
15. The Investigator considers the subject unlikely to comply with study procedures, restrictions, and requirements.

9.6 Restrictions during the study

Subjects must be willing to comply with the restrictions as outlined in Section 9.6.1 and 9.6.2.

9.6.1 General restrictions

1. Contraception requirements: Subjects of childbearing potential are expected to use contraceptive methods in accordance with inclusion criterion #5 or practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) during the clinical study.
2. Tobacco/nicotine products:

   In Part 1, subjects are only allowed to use their selected own brand snus product. The subjects are encouraged to follow their regular pattern of use.

   In Part 2, subjects shall abstain from any self-administered oral tobacco/nicotine products as well as smoking (cigarettes and e-cigarettes) for at least 12 hours prior to IP use visits and during each IP use visit (Visits 4-8).

   In Part 3, subjects are only allowed to use their selected NP product. During this part, subjects can adjust their usage patterns, using the products as much or as little as they wish.
3. Mouth related procedures: In Part 2, subjects shall abstain from eating, drinking, or conducting any other mouth related procedure (*e.g.*, tooth brushing) for 30 minutes prior to IP use, during IP use, and for 30 minutes after IP removal (Visits 4-8).
4. Drugs of abuse: Subjects shall abstain from any drugs of abuse during the study, *i.e.*, from screening (Visit 1) to the last visit (Visits 10).
5. Alcohol: Subjects shall abstain from alcohol for at least 12 hours prior to each visit from screening (Visit 1) to the last visit (Visits 10).
6. Blood donation: The subjects must not donate blood or plasma within 1 month of screening until 3 months after the last IP use visit of Part 2 (Visit 8).
7. Participation in other clinical studies: The subjects are not allowed to participate in any other clinical study from the screening visit (Visit 1) until the last visit (Visit 10).

9.6.2 Prior and concomitant therapy

Use of any prescribed medication that includes beta-adrenergic blocking agents (beta blockers) or ADHD medications, including *pro re nata* use, is not allowed from the screening visit (Visit 1) until the last visit (Visit 10). As outlined in exclusion criterion #12, subjects currently using beta-adrenergic blocking agents or ADHD medications will be excluded from participation in the study.

There will be no other restrictions concerning concomitant medications or therapies, as long as the subject is on a stable course of medication from the screening visit to the last IP use visit (Visit 10). Prescribed medications taken *pro re nata* may be a reason for exclusion as judged by the Investigator if they affect the subject's general condition and salivation.

As detailed in exclusion criterion # 8, a positive drug screen will exclude a subject from participation in the study. However, positive results that are expected given the subject's medical history and prescribed medications (*e.g.*, opioid analgesics) can be disregarded as judged by the Investigator. This does not include positive drug screens resulting from the use of beta-adrenergic blocking agents or ADHD medications.

9.7 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but do not fulfill all eligibility criteria and are not subsequently included in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects. Minimal information includes documentation of signed and dated informed consent form (ICF) and reason(s) for screening failure.

Re-screening can be performed if any of the following were reasons for screening failure or non-randomization, as judged by the Investigator:

- Practical reasons.
- Non-significant medical conditions (*e.g.*, influenza, nasopharyngitis).
- Plasma or blood donation outside of the allowed time windows.
- Reserve subjects.

For subjects who are re-screened, a new screening number will be assigned and new, signed ICF must be collected.

9.8 Subject withdrawal

9.8.1 General withdrawal criteria

Subjects are free to discontinue their participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up investigation or their future care. If possible, the reason for withdrawal of consent should be documented.

Subjects may be discontinued from the study at any time at the discretion of the Investigator.

Reasons for discontinuation can include:

- AE (as judged by the Investigator and/or Sponsor).
- Death.
- Logistical problem.
- Lost to follow-up.
- Non-compliance with study product.
- Non-compliance with study schedule.
- Physician decision.
- Pregnancy.
- Protocol deviation.

- Technical problems.
- Withdrawal of consent.
- Other.

9.8.2 Procedures for discontinuation of a subject from the study

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. Any ongoing AEs will be followed-up as described in Section 11.5.4.12.

The primary reason for discontinuation/early withdrawal must be specified in the eCRF and final drug accountability must be performed. If the reason for discontinuation was an AE, the AE must be specified in the eCRF.

9.8.3 Subject replacement

Subjects who are prematurely withdrawn prior to the start of Part 2 may be replaced at the discretion of the Sponsor.

9.9 Randomization (Part 2)

At Visit 4, subjects will be randomized to one of 10 use sequences using a Latin squares William's design balancing for first-order carry-over effects. The following sequences will be randomized:

Sequence 1: A B E C D

Sequence 2: B C A D E

Sequence 3: C D B E A

Sequence 4: D E C A B

Sequence 5: E A D B C

Sequence 6: D C E B A

Sequence 7: E D A C B

Sequence 8: A E B D C

Sequence 9: B A C E D

Sequence 10: C B D A E

where

A = NP 1 - 6 mg

B = NP 2 - 11 mg

C = NP 3 – 16.6 mg

D = Own brand snus product

E = Swedish pouch snus - 18 mg

As this is an open-label study, the IP use sequence to which each subject is allocated will be recorded in the eCRF. Computer-generated randomization lists for each site will be created using the statistical analysis software (SAS) Proc Plan, SAS Version 9.4. The randomization lists will contain site, subject number, randomization sequence, visit, and product.

The randomization list will be generated by CTC. The original randomization list will be kept by the randomizer and copies of the randomization list will be provided to each site and to the IP packing company.

10 INVESTIGATIONAL PRODUCTS

The IPs are supplied by Swedish Match.

10.1 Identity of test and comparator products

The test and comparator products that will be used in the study are detailed in Table 10.1-1.

Table 10.1-1 Identity of test and comparator products

IP	Nicotine content per pouch/portion
NP 1 – moist nicotine pouch product (Part 2, and option in Part 3)	6 mg
NP 2 – moist nicotine pouch product (Part 2, and option in Part 3)	11 mg
NP 3 – moist nicotine pouch product (Part 2, and option in Part 3)	16.6 mg
Subject's own brand snus (comparator) (Part 1 and Part 2)	optional
Swedish pouch snus General G.3 Extra Strong Slim (comparator) (Part 2)	18 mg

10.2 Manufacturing, packaging, and labelling

All IPs are manufactured and packaged by Swedish Match in compliance with the Swedish law on food production. Production sites and batch IDs for the IPs will be documented in the trial master file (TMF).

IPs will be transferred from the original container, weighed, and individually packaged in identical sealed food approved test containers at the Swedish Match analytical laboratory. For own brand loose snus, one unopened original container will be used. The containers will be labelled with unique identification numbers by Swedish Match in accordance with the randomization lists. IPs will be shipped by Swedish Match directly to CTC.

10.3 Conditions for storage

IPs will be stored in access-controlled storage areas at the study sites under refrigerator temperature (4-8°C).

10.4 Preparation and accountability

The IPs will be dispensed according to the randomization list by the site personnel. The Investigator will maintain a storage and accountability log as well as a dispensing log detailing the dates and quantities of study IPs received, and used by each subject, as well as any IPs destroyed at the end of the study. Products deliberately and/or accidentally destroyed by the site or the subject must be accounted for.

In Part 2, for users of loose snus, one unopened snus can, purchased by the Sponsor and shipped to CTC, will be weighed by the site personnel at CTC. The subjects will then take out as much snus as they normally use per portion. Finally, the snus can will be weighed again, and the weight difference will be documented.

10.5 Investigational product use instructions

In Part 1 and Part 3, the subjects will use their own brand snus product (Part 1) or the selected NP (Part 3) *ad libitum* throughout the 14-day period.

In Part 2, a single dose will be given in the morning of each IP use visit (Visits 4-8). Subjects will keep the pouch/portion between their upper lip and gum for 30 minutes and will be

instructed not to eat or drink, chew gum, or brush their teeth for 30 minutes before, during use, and 30 minutes after the use of IP.

Subjects shall abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for at least 12 hours prior to each IP use visit. To this end, subjects will be instructed to abstain from such products from approximately 20:00 (8 pm) the day before IP use visits (Visits 4-8). All IP uses will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

10.6 Investigational product final accountability

Any IP not used, as well as all empty containers, will be destructed at the site upon confirmation from the Sponsor. The Monitor will perform a final IP accountability reconciliation at the end of the study to verify that all unused IP is adequately destroyed and documented.

11 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of assessments are detailed in the schedule of events (Table 8.1-1 and Table 8.1-2).

11.1 Recording of data

The PIs will provide the Sponsor with all data produced during the study from the scheduled assessments. They will ensure the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the eCRF and in all required reports.

In Part 2, it is important that IP application and collection of PK blood sampling occurs as close as possible to scheduled time points in Table 8.1-2. In order to achieve this, the timing priority order at a particular timepoint is:

1. Blood samples for PK
2. Pulse rate assessment
3. Visual analogue scale (VAS) questions
4. MCQ

Allowed deviations from actual time points are outlined in Table 11.1-1 below.

Table 11.1-1 Allowed deviations from actual time points

Time point	IP use and collection	PK blood sampling (plasma)	Pulse rate (pulse oximeter)	VAS questions "craving" and "satisfaction"	VAS questions "product-liking" and "intent to use again"	MCQ
-00:15			± 5 min			
-00:10		Pre-use sample can be taken at any time within 10 min prior to IP use[1].		± 3 min[2]		
-00:01						
00:00	± 0 min (IP admin.)					
00:05		± 2 min	± 2 min	± 3 min		
00:10		± 2 min	± 2 min	± 3 min		
00:15		± 2 min	± 2 min	± 3 min		
00:20		± 2 min	± 2 min	± 3 min		
00:30	±1 min (IP collection)	± 5 min	± 5 min	± 5 min	± 10 min	± 10 min
00:40		± 5 min	± 5 min	± 5 min		
01:00		± 10 min	± 10 min	± 10 min		
01:30		± 10 min	± 10 min	± 10 min		
02:00		± 10 min	± 10 min	± 10 min		
04:00		± 10 min	± 10 min	± 10 min		
06:00		± 10 min	± 10 min	± 10 min		

1. Pre-use PK blood sample should not be taken in direct association with the pre-use pulse rate assessment at -15 min.
2. Only "craving" at -10 min.

11.2 Electronic patient reported outcome

To fill in the eDiary, the subjects themselves will record data using an electronic patient reported outcome (ePRO) system (ViedocMe™) linked to the eCRF. The ePRO system includes password protection and internal quality checks.

The subjects will receive eDiary training and instructions at Visit 2 (reminder if needed on Visit 9). The subjects will be instructed to record eDiary data once daily (for the previous calendar day) during the 14-days *ad libitum* use periods of Part 1 and 3. The following data will be collected:

- the number of pouches/portions used
- estimated average use time for each pouch/portion (in minutes)
- if a new box of pouches/loose snus was opened that day (yes/no)

Text reminders to fill in the eDiary will be sent through ViedocMe™. The site personnel will perform an eDiary compliance check when the subject returns to the site on Visit 3 and 10. All ePRO data will be stored together with the eCRF data.

11.3 Demographics and other baseline characteristics

11.3.1 Informed consent

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 14.3.

11.3.2 Demographic information

The following demographic data will be recorded: gender, age, ethnicity, and race.

11.3.3 Medical/surgical history

Medical/surgical history will be obtained by subject interview in order to verify that the eligibility criteria are met.

11.3.4 History of nicotine use and currently used products

History of oral tobacco/nicotine products use in terms of brands, average consumption per day during the last 30 days, and duration of use (years, months), history of smoking in terms of number of cigarettes per day during the last 30 days, as well as history of vaping in terms of number of days during last 30 days, will be obtained by subject interview.

The subject's current Swedish snus product use in terms of brand, flavor, and strength will be recorded at the screening visit. The subject will select one Swedish snus pouch or loose snus product that they will exclusively use during Part 1 of the study. The chosen product should be the brand, nicotine strength, and flavor variation predominantly used in the past month, unless no single brand was used exclusively. For Part 2 of the study and for chemical analyses, the Sponsor will purchase the selected snus product.

11.3.5 Eligibility criteria

Eligibility criteria should be checked during screening and verified before randomization and use of the first IP on Visit 4. The criteria are specified in Sections 9.4 and 9.5.

11.3.6 Height, weight, and body mass index

Weight and height will be measured without shoes. BMI will be calculated, with one decimal, from the recorded height and weight.

11.3.7 Physical examination

A brief physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes, and extremities.

11.3.8 Vital signs

Systolic and diastolic blood pressure, and pulse will be measured at screening in supine position after 10 minutes of rest.

Pulse rate assessments will be considered as ”normal” if within the following ranges:

-At screening (Visit 1): 40 to 90 beats per minute (bpm)

-During IP use visits (Visit 4 to Visit 8): 40 to 100 bpm. Refer to Section 11.5.3.1.

11.3.9 Electrocardiogram

Single 12-lead ECGs will be recorded at screening in supine position after 10 minutes of rest using an ECG machine. The resting HR and PQ/PR, QRS, QT and QTcF intervals will be recorded.

ECGs will be reviewed and interpreted on-site by the Investigator.

11.3.10 HIV and hepatitis B/C

Subjects will be tested for HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen, hepatitis B virus surface antigen, and hepatitis B and C virus antibodies prior to inclusion into the study. Any positive result will exclude the subject from participating in the study.

11.3.11 Pregnancy test

All females of childbearing potential will do a urine pregnancy test at the screening visit and at visits specified in Table 8.1-1 (urine dipstick). At Visits 5 to 9, pregnancy tests (urine dipstick) will be performed only at the discretion of the Investigator.

11.3.12 Urine drug screen

Urine will be screened for drugs of abuse at time points outlined in the schedule of events (Table 8.1-1) using the Drug Screen Multi-15 Dip Test. Additional random tests can be performed during the study period, at the discretion of the Investigator.

11.3.13 Alcohol test

An alcohol test will be performed at time points outlined in the schedule of events (Table 8.1-1 and Table 8.1-2). Additional random tests can be performed during the study period, at the discretion of the Investigator.

11.3.14 Baseline symptoms

A baseline symptom is defined as an event that occurs between the subject’s signing of the ICF until the first use of IP in Part 2 (*i.e.*, an event that occurs during the screening period).

Such events are not AEs but will be recorded as baseline symptoms in the AE Log in the eCRF.

11.3.15 Prior and concomitant medication

Prior medications taken within 2 weeks prior to screening will be obtained by subject interview in order to verify that the eligibility criteria are met.

Medications are classified as prior if the stop date was before or on the day of the first IP use (prior to IP use) in Part 2, and as concomitant if ongoing on the day of the first IP use in Part 2, stopped after the first IP use in Part 2, or started after the first IP use in Part 2. To distinguish between prior and concomitant medications on the first IP use visit, the start time of any newly introduced medication or the stop time of any previously ongoing medication must be recorded in the eCRF.

Any use of prior/concomitant medication from the screening visit until the last visit (Visit 10) must be documented appropriately in the subject's eCRF. Relevant information (*i.e.*, name of medication, dose, dose form, unit, route, frequency, start and stop dates, and reason for use) must be recorded. All changes in medication must be noted in the eCRF.

11.3.16 Fagerström test for nicotine dependency

At the screening visit, to confirm the subjects' level of nicotine dependency, the subjects will complete the Fagerström nicotine dependency test (for snus use) on paper. The study personnel will enter the test scores in the eCRF.

11.4 Assessments related to primary endpoints

11.4.1 Nicotine plasma concentration and pharmacokinetic sampling and analysis

In Part 2, venous blood samples (approximately 3 mL) for the determination of plasma concentrations of nicotine after IP use will be collected through an indwelling venous catheter at the pre-specified visits and time points detailed in Table 8.1-1 and Table 8.1-2. It is important that blood PK sampling does not deviate from the planned time points more than the allowed time deviations, as outlined in Table 11.1-1.

Pre-PK sampling should be conducted -10 minutes to -1 minute before the first IP use.

The date and time of collection of each sample will be recorded in the eCRF.

The blood samples will be collected in pre-labelled tubes. All the collected blood samples will be centrifuged for 10 minutes at 20°C (± 4°C) to separate the plasma within 60 minutes from when the sample was drawn. The separated plasma from each blood sample will be divided into 2 aliquots in pre-labelled cryotubes and frozen at -80°C within 1 hour after the centrifugation. Further details will be described in a separate laboratory manual.

Plasma samples for determination of plasma concentrations of nicotine will be analyzed by Lablytica Life Science AB, Uppsala, Sweden, by means of a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The details of the analytical method used will be described in a separate bioanalytical report.

11.5 Assessments related to secondary endpoints

11.5.1 Pharmacokinetic sampling related to secondary endpoints

PK sampling related to secondary endpoint 2 is part of the procedure described in Section 11.4.1 above.

11.5.2 Nicotine extraction from pouches/portions

Used pouches/portions will be collected after 30 minutes (± 1 min) of use for the determination of residual nicotine in the IPs.

All the collected pouches/portions will be frozen within 60 minutes at -20°C. Pouches/loose snus for extraction of nicotine will be analyzed by Swedish Match.

In Part 2, all pouches/portions will be weighed prior to use, as will reference pouches/portions.

11.5.3 Pharmacodynamic effects

The PD effects of the IPs will be assessed by measuring pulse rate and subjective parameters (using VAS and MCQ) at pre-defined time points as described below and in Table 8.1-2.

11.5.3.1 Pulse rate

The pulse rate will be monitored using a pulse oximeter and will be spot-assessed at -15 min pre-use, as well as at 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h, and 6 h post-use (see Table 8.1-2). Any post-IP measurements of pulse rate outside of normal ranges will be judged as clinically significant or not clinically significant. The assessment will be recorded in the eCRF. Abnormal post-IP use findings assessed by the Investigator as clinically significant will be reported as AEs. Refer to Section 11.3.8.

Allowed deviations from actual time points are outlined in Table 11.1-1.

11.5.3.2 Subjective parameters

Subjective parameters (“craving”, “satisfaction”, “product-liking”, and “intent to use again”) will be rated using VAS, anchored with “not at all” to “extremely”, or “very likely” for the “intent to use again” parameter.

The “craving” parameter will be assessed with the question “Right now, how strong is your urge to snus?” at the same pre-defined time points as the pulse rate assessments: -10 min prior to IP use, and 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h, and 6 h post-use.

The “satisfaction” parameter will be assessed with the question “Right now, is the product satisfying?” at the pre-defined time points: 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h, and 6 h post-use.

In addition, “product-liking” and “intent to use again” parameters will be assessed at 30 minutes post-use with the questions “How much did you like the product?” and “How likely are you to use this product again in the future?”, respectively.

Time points for each of the VAS questions are outlined in Table 8.1-2, and allowed deviations from actual time are outlined in Table 11.1-1.

11.5.4 Adverse events

The PIs are responsible for ensuring that all medical staff involved in the study are familiar with the content of this section and the content of the CTC standard operating procedures (SOPs) regarding emergencies.

AEs will be handled in accordance with applicable regulations and guidelines [16].

For the purpose of this study, AEs will be assessed in relation to the IPs and comparators starting from the first IP use of Part 2.

11.5.4.1 Definition of adverse event

An AE is defined as any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this IP use.

An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

11.5.4.2 Definition of serious adverse event

An SAE is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,

  NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in various situations. These situations may include "important medical events" that, while not immediately life-threatening or resulting in death or hospitalization could jeopardize the subject’s well-being or require intervention to prevent any of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

11.5.4.3 Time period and frequency for collecting adverse events

All AEs (including SAEs) will be collected from the start of first IP use in Part 2 until the last visit (Visit 10).

Any AE with start date on the day of each IP use must be recorded with start time.

On the last IP use visit (Visit 10), information on new AEs or SAEs, if any, and stop dates for ongoing events must be recorded as applicable.

Investigators will not be obliged to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and they consider the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

11.5.4.4 Collection of adverse events

AEs identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject.
- AEs observed by the Investigator or medical personnel.
- AEs elicited based on non-leading questions from the Investigator or medical personnel.

11.5.4.5 Recording of adverse events

AEs must be recorded in the AE Log of the eCRF. The Investigator must provide information on the AE, preferably as a diagnosis or at least as signs and symptoms; start and end dates, start and end time; intensity; causal relationship to IP and comparators, action taken, and outcome. If the AE is serious, this must be indicated in the eCRF.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

11.5.4.6 Assessment of seriousness

The Investigator must assess and document the seriousness (serious or non-serious) of each AE using the definitions in Section 11.5.4.2. If the event is assessed as serious it must be reported as an SAE by the Investigator to the Sponsor according to Section 11.5.4.10.

For the seriousness criteria of inpatient hospitalization or prolongation of existing hospitalization to be fulfilled, the AE requires at least an overnight admission (24 hours) or prolongs a hospitalization beyond the expected length of stay. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes.

Planned hospitalizations or surgical interventions for a condition that existed before the subject signed the ICF, and that did not change in intensity, are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative approach will be taken, and the AE will be reported as an SAE.

11.5.4.7 Assessment of intensity

The grading of the intensity of AEs will follow the common terminology criteria for adverse events (CTCAE) v5.0 [17]. Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.

The Investigator must assess the intensity of an AE using the following definitions, and record it in the AE Log of the eCRF:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local, or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.

Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self- care ADL**.
Grade 4	Life-threatening consequences: urgent intervention indicated.
Grade 5	Death related to AE.

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc.*

**Self-care ADL refers to bathing, dressing, and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.5.4.8 Assessment of causal relationship

The Investigator must assess the causal relationship between an AE and the use of the IP and comparators using the definitions below. Each assessment should be recorded in the AE Log of the eCRF.

Probable	The event has a strong temporal relationship to the IP and comparators, or recurs on re-challenge, and another etiology is unlikely or significantly less likely.
Possible	The event has a suggestive temporal relationship to the IP and comparators, and an alternative etiology is equally or less likely.
Unlikely	The event has no temporal relationship to the IP and comparators or is due to underlying or concurrent illness or effect of another drug (*i.e.*, there is no causal relationship between the IP, and the event).

An AE is considered causally related to the use of the IP and comparators when the causality assessment is probable or possible.

11.5.4.9 Outcome of adverse event

The Investigator must assess the outcome of an AE using the definitions below and record it on the AE Log of the eCRF.

Recovered/resolved	The subject has recovered completely, and no symptoms remain.
Recovering/resolving	The subject's condition is improving, but symptoms still remain.
Recovered/resolved with sequelae	The subject has recovered, but some symptoms remain (*e.g.*, the subject had a stroke and is functioning normally, but has some motor impairment).
Not recovered/not resolved	The subject's condition has not improved, and the symptoms are unchanged (*e.g.*, atrial fibrillation has become chronic).
Fatal	
Unknown	

11.5.4.10 Action taken with investigational product

The Investigator must document the action taken with the IP using one of the options “Dose not changed”, “Drug interrupted”, “Drug withdrawn”, “Not applicable”, or “Unknown”, and record it on the AE Log of the eCRF.

11.5.4.11 Reporting of serious adverse events

The Investigator must report SAEs within **24 hours** of awareness to the Sponsor or its designee, this includes both initial information and any subsequent relevant/significant follow-up information to a previously reported SAE. The primary mechanism for reporting an SAE will be via the eCRF. When the Investigator classifies the event as “serious” in the eCRF, and signs off the event, an automatic e-mail alert is sent to the Sponsor or its designee, and any other pre-defined recipients.

The backup procedure for reporting an SAE in case the eCRF is unavailable, will be via the paper SAE form provided in the Investigator Site File (ISF). The Investigator must fill in the SAE form and send it Sponsor or its designee. The study site must notify the site Monitor via phone or e-mail about the submission of the SAE report. As soon as the site personnel have access to the eCRF, the SAE must be reported electronically as well. The completed, signed, and dated paper SAE form should, within 24 hours, be scanned and delivered via encrypted e-mail or secure file transfer to:

[redacted]

A copy of the SAE paper form must also be delivered via encrypted e-mail or secure file transfer to CTC at: [redacted]

All available information regarding the SAE must be entered in the AE Log for the specific subject, *i.e.*, AE term, intensity, causality, outcome, seriousness criteria, action taken with IP, a narrative including the Investigators rationale for the causality assessment.

The SAE report will be reviewed by the Sponsor or its designee to ensure that the report is valid. The Sponsor or its designee will acknowledge receipt of the SAE report to the reporting Investigator. For SAEs where important or relevant information is missing, follow-up queries to the site are raised promptly to keep the regulatory reporting timelines.

Sponsor will perform an independent assessment of causality, including a rationale for the assessment. The causality assessment given by the Investigator should not be downgraded by the Sponsor. If the Sponsor disagrees with the Investigator’s causality assessment, the opinion of both the Investigator and the Sponsor should be provided with the report.

If any additional information or documentation (*e.g.*, autopsy report) on the SAE is required for Sponsor’s assessment of the SAE, the Sponsor or its designee will request this information from the Investigator, and the Investigator is required to promptly respond to the request.

Any subsequent relevant/significant follow-up information to a previously reported SAE must be entered in the AE Log for the specific subject. If the Investigator makes any changes to the assessment of the case *e.g.*, changes in seriousness, causality, or intensity, a justification for the change should be provided in the case narrative. If the SAE report in the eCRF is updated, a new automatic e-mail alert is sent to Sponsor or its designee.

Detailed information on the SAE handling will be described in a study specific safety management plan.

11.5.4.12 Treatment and follow-up of adverse events

Subjects with AEs that occur during the study must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed-up until resolution or to the last visit (Visit 10), whichever comes first. On the last visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded (if known). AEs assessed as stable by the Investigator at the last IP use visit will not have to be followed-up until resolution.

It is the responsibility of the Investigator to follow-up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

11.5.4.13 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the study IP use must be stopped immediately, and the subject should be withdrawn from the study.

Pregnancy itself will not be regarded as an AE unless there is a suspicion that the IP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed-up by the Investigator and the Sponsor and documented even after the subject was discontinued from the study.

All pregnancies must immediately be reported by the Investigator to the Sponsor or designee using the Pregnancy Report Form provided in the ISF. Once known, information on the outcome of the pregnancy must also be reported to the Sponsor or designee using the Pregnancy Report Form.

All events of congenital abnormalities, birth defects and spontaneous miscarriages are SAEs and must be handled and reported as such as described in Section 11.5.4.10.

11.5.4.14 Treatment of overdose

An overdose is a dose in excess of the dose specified for subject in this clinical study protocol (CSP).

Overdosing is not likely to occur in this study since all IPs will be administered as single doses by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures will be adopted as required.

Overdoses must be documented in the eCRF. An overdose associated with AE will be recorded as the AE diagnosis/symptoms in the AE Log of the eCRF. An overdose without associated symptoms will only be reported in the subject's medical records and documented in the PD log.

11.6 Assessments related to exploratory endpoints

11.6.1.1 Exploratory subjective parameters

The subjective parameter "product-liking" *vs*. the subject's usual snus/nicotine product of choice will be assessed at 30 minutes with the MCQ "How much did you like the product compared with your usual snus or nicotine product of choice?" on a 3-point scale:

"1 = To a lesser extent, 2 = To the same extent, 3 = To a greater extent."

The allowed deviation from actual time for the MCQ is outlined in Table 11.1-1.

11.6.1.2 Nicotine and tobacco-specific nitrosamines extraction related to exploratory endpoints

Nicotine extraction related to exploratory endpoint 2 is part of the procedure described in Section 11.5.2 above.

For Part 1 and 3, nicotine extraction (related to exploratory endpoints 6 and 7) will be calculated from analysis of used pouches/loose snus collected by the subject during *ad libitum* use of the subject's own brand Swedish snus (Part 1) and the selected NP product (Part 3).

Used pouches/loose snus will be shipped frozen to Swedish Match for chemical analysis of the extracted amount of nicotine, NNK, and NNN. The Sponsor will purchase the applicable products used by the subjects in Part 1 for chemical characterization of the unused pouches/loose snus at Swedish Match. For pouched products, the calculation of the extracted amount and fraction of nicotine, NNK, and NNN will involve subtracting the average of the pouches used by the subjects from the average of 10 unused pouches. For loose snus users, the calculation is based on a single unused reference portion. The weight of this reference portion is identical to the portion that each user prepares themselves during Part 2.

11.6.1.3 Ad libitum pattern of use

For Part 1 and 3, *ad libitum* use (related to exploratory endpoint 3) will be analyzed from eDiary data provided by the subject for a 14-day period during *ad libitum* use of the subject's own brand Swedish snus (Part 1) and the selected NP product (Part 3).

The subject will document their consumption via an eDiary (ViedocMe) during a 14-day study period (see Section 11.2) starting after Visit 2 (Part 1) and the day after Visit 9 (Part 3). Electronic diary compliance will be checked by the study personnel upon return to the study site at Visits 3 and 10.

11.6.1.4 Pharmacokinetic sampling related to exploratory endpoints

PK sampling related to exploratory endpoints 2 and 4, is part of the procedure described in Section 11.4.1 above.

11.6.1.5 Urine sampling for analysis of biomarkers of exposure

In Part 1 and Part 3, urine will be collected for 24 hours at the end of the 14-day *ad libitum* use period (see Table 8.1-1) for analysis of BoE in urine.

Following bladder emptying after arrival at the study site in the morning, the 24-hour urine collection interval will be started. Each void will be added to the 24-hour collection. The total volume of urine in the end of the 24-hours will be determined by total weight and documented

in the eCRF. The date and time interval of the collection (start and stop time) will be recorded in the eCRF (allowed time window: ±15 minutes).

Two urine aliquots (2 mL) from the collection interval will be transferred to pre-labelled polypropylene cryotubes and will thereafter immediately be frozen at -80°C.

Details on sample collection and handling will be provided in a separate laboratory manual.

Samples for determination of urine concentrations of BoE (nicotine and its metabolites, NNN, NNAL, and anabasine) will be analyzed by Lablytica, Uppsala, Sweden, by means of a qualified bioanalytical method. In addition, creatinine will be assessed for normalization of urinary BoE concentrations. The details of the analytical method used will be described in a separate bioanalytical report.

11.7 Appropriateness of measurements

All methods used are commonly used in standard medical care and in phase I clinical studies. Non-compartmental analysis of PK parameters is standard for phase I clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

The sample collection procedure for PK analysis is described in Section 11.4.1.

12.2 Volume of blood

The anticipated volume of blood samples collected from screening until Visit 8 from each subject will be approximately 184 mL (Table 12.2-1). For reference, a regular blood donation consists of between 350 mL to 450 mL (±10%) and is typically collected in a single occasion for persons weighing at least 45-50 kg [18]. Additional blood samples for safety evaluation may be collected at the discretion of the Investigator.

Table 12.2-1 Estimated blood volumes

Assessment	Estimated number of sampling occasions	Estimated volume per occasion (mL)	Total (mL)
PK blood sampling	60	3 mL	180 mL
HIV, Hepatitis B/C	1	4 mL	4 mL
		Total:	**184 mL**

12.3 Handling, storage, and destruction of laboratory samples

All biological samples will be registered in a biobank at CTC (Swedish Health and Social Care Inspectorate biobank registry number 893).

Any remains from the laboratory samples will be disposed of after analyses.

The samples for analyses of plasma PK parameters and urine biomarkers will be stored at ≤ -80 °C until analyzed. The samples will be disposed of after the clinical study report (CSR) has been finalized.

12.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

CTC keeps full traceability of collected biological samples from the study subjects while in storage at the study site until shipment and keeps documentation of receipt of arrival. The sample receiver (the analytical laboratory) will keep full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor will keep oversight of the entire lifecycle of the samples through internal procedures, monitoring of study sites and auditing of external laboratory providers.

12.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of biological samples donated, the samples will be disposed of/destroyed, if not already analyzed and documented.

The PI will ensure that:

- Subject withdrawal of consent is notified immediately to the Sponsor.
- Biological samples from the subject, if stored at the study site, are immediately identified, disposed of/destroyed and the action is documented.

The Sponsor has to ensure that the laboratory/laboratories holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the study site and the action is documented.

13 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

13.1 Quality management: critical processes, systems, and data identification

During CSP development, the Sponsor will identify those processes, systems (facilities, computerized systems) and data that are critical to ensure human subject protection and the reliability of study results according to applicable SOPs and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6(R2) guideline [19].

Identified risks will be categorized separately from the CSP.

13.2 Quality assurance and quality control

The Sponsor has delegated the responsibilities outlined below to CTC while maintaining overall study oversight:

1. Implementing and maintaining quality assurance and quality control (QC) systems with written SOPs with regard to management of identified risks, CSP compliance, good clinical practice (GCP) compliance and applicable regulatory requirements.
2. Securing agreements with involved subcontractors and performing regular subcontractor oversight to ensure CSP compliance, GCP compliance and compliance with applicable regulatory requirements.
3. Implementing a risk based validated EDC system and maintain SOPs for the whole life cycle of the system.
4. QC application to each stage of data handling to ensure that all data are reliable and have been processed correctly.

14 ETHICAL AND REGULATORY REQUIREMENTS

14.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki [20] and are consistent with the ICH E6 (R2) guideline for GCP [19], applicable sections of the Clinical Trials Regulation European Union (EU) no.536/2014 [16], and applicable local regulatory requirements.

14.2 Ethics and regulatory review

The coordinating PI is responsible for submission of the CSP, the subject information and ICF, any other written information to be provided to the subjects, and any advertisements used for recruitment of subjects to applicable IEC for approval.

Approval must be obtained in writing from the IEC before the first subject can be recruited.

The Sponsor will provide the IEC and PI with safety updates/reports according to local requirements.

14.3 Subject information and consent

It is the responsibility of the Investigator or an authorized associate to give each potential study subject adequate verbal and written information before any study specific assessments are performed.

The information will include the objectives and the procedures of the study as well as any risks or inconvenience involved. It will be emphasized that participation in the study is voluntary and that the subject may withdraw from participation at any time and for any reason, without any prejudice. All subjects will be given the opportunity to ask questions about the study and will be given sufficient time to consider participation before signing and dating the ICF.

Before performing any study related procedures, the ICF must be signed and personally dated by the subject and by the Investigator. A copy of the subject information including the signed and dated ICF will be provided to the subject.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the eCRF. The subject information sheet and the signed and dated ICF should be filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

14.4 Subject privacy and data protection

The clinical personnel affirm and uphold the principle of the subject’s right to privacy during and after the study.

The ICF includes information that data will be recorded, collected, and processed and information related to potential transfer to European Economic Area (EEA) or non-EEA countries. In accordance with the General Data Protection Regulation (GDPR [EU] 2016/679) [21], these pseudonymized data will not identify any persons taking part in the study. If any part of the data is handled by any other organization, inside or outside the EU, appropriate agreements and/or other documentation will be established, to ensure that the data processing is performed in accordance with the provisions of the GDPR and other relevant legislation before any data transfer takes place.

The potential subject should be informed that by signing and dating the ICF they approve that the authorized representatives from the Sponsor and CTC, as well as the concerned IEC, have direct access to their medical records for verification of clinical study procedures. For further details on the subject information and ICF process, refer to Section 14.3.

The subject has the right to request access to their personal data and the right to request rectification of any data that is not correct and/or complete in accordance with GDPR [21] and the request will be raised to the PIs.

The Investigator must file a subject identification list which includes sufficient information to link records, *i.e.*, the eCRF and clinical records. This list must be preserved for possible future inspections/audits but must not be made available to the Sponsor except for monitoring or auditing purposes by the authorized representatives from the Sponsor.

Personal data that are collected in the study such as health information and ethnicity are considered as sensitive personal data. This data will be pseudonymized, *i.e.*, personally identifiable information will be removed and replaced by a unique subject ID and will be processed by the Sponsor and other involved parties during the study. After the study ends, only pseudonymized data, *i.e.*, aggregated data sets, can be used.

For this study, the Sponsor is the data controller of all data processed during the study (*e.g.*, TMF and study reports) and CTC AB is the data processor. Any subcontractors used in the study are also data processors.

For data that are processed at the clinic(s) (*e.g.*, medical records and ISF), CTC AB is the data controller.

14.5 Changes to the approved clinical study protocol

Any proposed change to the approved final CSP, including appendixes, will be documented in a written and numbered CSP amendment. All substantial amendments to the CSP must be approved by the appropriate IEC before implementation according to applicable regulations.

14.6 Audits and inspections

Authorized representatives of the Sponsor, or an IEC may perform audits or inspections at the study site, including source data verification (SDV). The purpose of an audit or inspection is to examine all investigation-related activities and documents systematically and independently, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the CSP, ICH-GCP guidelines, and any applicable regulatory requirements.

14.7 Insurance

Subjects will be covered under the Sponsor’s liability insurance policy through IF insurances. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. CTC has a company insurance covering medical procedures and services performed by CTC. The certificate of insurance can be provided upon request.

15 STUDY MANAGEMENT

15.1 Training of study site personnel

Before inclusion of the first study subject, a Sponsor representative or delegate will perform a study initiation visit at the study sites. The requirements of the CSP and related documents will be reviewed and discussed, and the investigational staff will be trained in any study specific procedures and system(s) utilized.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A Curriculum Vitae will be available for all staff delegated study specific duties.

15.2 Clinical monitoring

The Sponsor is responsible for securing agreement from all involved parties to ensure direct access to all participating sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by domestic and foreign regulatory authorities.

As defined in the risk-based monitoring (RBM) plan, approved by the Sponsor and provided separately, the responsible Monitor will periodically visit the study sites at times agreed upon by the Investigator and the Monitor. At each monitoring visit, the role of the Monitor is (but not limited to) the following:

- provide information and support to the investigational team,
- confirm that facilities and resources remain acceptable,
- confirm that the investigational team is adhering to the CSP, applicable SOPs, guidelines, manuals, and regulatory requirements,
- verify that data are being accurately and timely recorded in the eCRFs and that IP accountability checks are being performed,
- verify that data in the eCRF are consistent with the clinical records (SDV) in accordance with the RBM plan,
- verify that the correct informed consent procedure has been adhered to for participating subjects,
- ensure that withdrawal of informed consent to the use of the subject’s biological samples will be reported and biological samples are identified and disposed of/destructed accordingly, and that this action is documented and reported to the subject,
- verify that AEs are recorded and reported in a timely manner and according to the CSP,
- raise and escalate any serious quality issues, serious GCP breach and any data privacy breach to the Sponsor.

Centralized monitoring will also be performed continuously by project team members at CTC in accordance with the RBM plan.

When the study has been completed, all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

15.3 Source data documents

A separate origin of source data list will be generated before the start of enrollment, specifying the location of the source of derived information appearing in the eCRF. This document must be signed by the PI and the Monitor to confirm agreement before the start of recruitment.

Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, and that verify the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the study. They include laboratory notes, memoranda, material dispensing records, subject files, *etc*. The eCRF may constitute source data if clearly defined in the origin of source data list.

The Investigator must guarantee access to source documents to the Monitor, and the IECs, if required.

15.4 Study agreements

This study is fully financed by the Sponsor, Swedish Match. The management and conduct of the clinical study have been outsourced to the contract research organization (CRO), CTC. The coordinating PI and the PI at each site are employees of CTC.

The agreements between Sponsor and CTC must be in place before any study related procedures can take place, or subjects be enrolled.

The Sponsor and CRO responsibility and duty split is regulated in a separate clinical study agreement. The PIs must comply with all the terms, conditions, and obligations of the clinical study agreement for this clinical study.

15.5 Study timetable and end of study

The study is expected to start in Q1 2025 and to be completed by Q4 2025.

A subject is considered to have completed the study if they have completed all visits in the study including the last visit. The end of clinical part of the study is defined as the date of the last visit of the last subject in the study.

The end of the study period is defined as the date of the final CSR.

15.6 Termination of the study

The Investigator or the Sponsor may terminate this study prematurely for any reasonable cause. The IEC must be informed promptly. Conditions that may warrant study termination include, but are not limited to, a decision by the Sponsor to suspend or discontinue the development of the IP.

If the study is prematurely terminated or suspended for any reason, the Investigator must promptly inform the study subjects and must assure appropriate follow-up for the subjects.

15.7 Reporting and publication

15.7.1 Clinical study report

After completion of the study, an ICH E3 [22] guideline-compliant CSR describing the conduct of the study, any statistical analyses performed, and the results obtained will be prepared by the Sponsor or their designee. The CSR will be reviewed and approved by, at a minimum, the PI, the Statistician, and the Sponsor.

All results obtained from any exploratory analyses may be reported separately.

15.7.2 Confidentiality and ownership of study data

Any confidential information relating to the IPs or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the study are responsible for protecting the confidentiality of this proprietary information.

15.7.3 Publication

The results from this study may be submitted for publication at the discretion of the Sponsor.

15.8 Archiving

The PI is responsible for maintaining essential documents, (as defined in ICH E6(R2), Section 8 [19]) for 25 years after finalization of the CSR. This includes any original source documents related to the study, the subject identification list (providing the sole link between named subject source records and pseudonymous eCRF data), the original signed ICFs and detailed records of IP disposition.

It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

The Sponsor will archive the TMF in accordance with the ICH E6(2) guideline, Section 8 [19], and applicable regulatory requirements.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the study site and filed in the ISF for archiving for 25 years after finalization of the CSR.

The completed original eCRFs are the sole property of the Sponsor and must not be made available in any form to third parties, except for authorized representatives of appropriate health/regulatory authorities, without written permission from the Sponsor.

16 DATA MANAGEMENT

The data management routines include procedures for handling of the eCRF, database setup and management, data entry and verification, data validation, QC of the database, and documentation of the performed activities including information of discrepancies in the process. The database, data entry screens, and program will be designed in accordance with the CSP.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of computerized online edit checks identifying *e.g.*, data values that are outside the allowed range and SAS-programmed batch checks on data exports. All study specific and standard data validation programming will be tested prior to being used on final data.

Detailed information on data management will be described in a study specific Data Management Plan.

16.1 The web-based eCRF

Clinical data will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by Viedoc Technologies AB. The eCRF includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents or at bedside (if the eCRF data constitutes source data). Source data are to be defined at the sites before inclusion of the first subject (Section 15.3).

Authorized site personnel designated by the Investigator will complete data collection. Appropriate training and security measures will be completed with the Investigator and all authorized study site personnel prior to the study being initiated and any data being entered into the system for any study subject.

16.2 The entering of data into the eCRF

All entries, corrections, and alterations are to be made by the Investigator or designee. Neither the Monitor nor any other study team member besides site staff can enter data in the eCRF. All data should be entered in English. The eCRFs should be completed as soon as possible during or after the subject's visit. To avoid inter-observer variability, every effort should be made to ensure that preferably same individual who made the initial baseline determinations completes all corresponding follow-up evaluations. The Investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available, not applicable, or unknown, the Investigator or assigned clinical staff will record such information in the eCRF.

The Investigator will be required to electronically sign off on the clinical data. This will be performed by means of the Investigator's unique User ID and password; date and time stamps will be added automatically at time of electronic signature.

16.3 The query process

The Monitor will review the eCRFs and evaluate them for completeness and consistency. Data in the eCRF will be compared with the respective source documents to ensure that there are no discrepancies for critical data as described in the RBM plan. All entries, corrections, and alterations are to be made by the Investigator or designee.

If corrections are needed, queries will be raised within the eCRF, either as a result of built-in edit checks or manually raised by the Monitor. An appropriate member of the site staff will answer the queries in the eCRF either by correcting the data or by entering a response to the query.

16.4 Audit trail

All entries in the eCRF will be fully recorded in a protected audit trail. Once clinical data have been saved, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who made the change, together with time and date will be logged.

16.5 External data

External data consists of data that are not recorded in the eCRF. Data may be received in electronic format. Key variables are defined in order to uniquely identify each sample record. File and data formats are agreed with the external data provider.

16.6 Medical coding

Medical coding will be performed by trained personnel at CTC. AEs and medical/surgical history verbatim terms are coded using the medical dictionary of regulatory activities (MedDRA, latest version available at eCRF setup).

Prior and concomitant medications will be coded according to the World Health Organization (WHO) anatomic therapeutic chemical (ATC) classification system (latest version available at eCRF setup). All coding will be approved by the Sponsor prior to database lock.

16.7 Database lock

When all data has been entered and discrepancies solved, clean file will be declared, the database will be locked, and the data will be analyzed.

17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate statistical analysis plan (SAP), which will be signed and approved prior to database lock.

17.1 General

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum, and maximum value, Q1-Q3 (interquartile range [IQR]).

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by IP, and by assessment time. Individual subject data will be listed by subject number, IP, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC, US). The PK parameters will be calculated by non-compartmental analysis using the software Phoenix WinNonlin® version 8.3 or later (Certara Inc., Princeton, NJ, US).

For Part 2, baseline will be defined as the last data collection point prior to each IP use.

No adjustment for multiple comparisons will be performed. All formal comparisons will be made toward a designated reference product and all significant findings will be reviewed for medical relevance.

17.1.1 Missing, unused and spurious data

Generally, no imputation of data will be performed. In case of missing start and stop times of AEs that cannot be investigated further, missing data will be imputed according to a worst-case scenario, *i.e.*, start time will be imputed as the closest time point post intake of IP and end time as 23:59, resulting in the longest possible treatment emergent duration of the AE.

Spurious data will be evaluated continuously through data validation (see Section 16) and, if connected to protocol deviations, evaluated case-by-case at the latest prior to declaration of clean file and database lock.

Deviations from the original SAP will be described in the CSR.

Methods for handling of missing, unused, and spurious data will be further specified in the SAP.

17.2 Determination of sample size

The study will include approximately 53 subjects with the aim of randomizing at least 53 subjects in Part 2 and achieving 45 evaluable subjects (for Part 2).

The sample size has been calculated considering the primary endpoint: to compare the exposure in terms of AUC_{inf} between the NP 11 mg and the comparator, Swedish snus 18 mg. Using a coefficient of variation (CV) of 33 %, based on previous studies, a power of 90%, a significance level of 5% (as judged by a 95% confidence interval) and an upper non-inferiority bound for the back-transformed geometric means ratio of 1.25 (NP 11 mg/comparator), 45 evaluable subjects will be needed. Assuming a dropout rate of 15%, a total of 53 subjects will be randomized.

Refer to Section 17.6.1 for a full description of the envisioned primary analysis model.

Efforts will be made to include at least 21 female subjects (approximately 40%). However, a minimum of 11 female subjects (approximately 20%) will be considered acceptable. Additionally, efforts will be made to include 10 to 15 regular users of loose snus. These subjects will use a loose snus of their choice as their own brand product during both Part 1 and Part 2.

17.3 Analysis data sets

17.3.1 Full analysis set

The Full analysis set (FAS) will consist of all subjects who have been randomized and used at least 1 IP. This population will be used for the safety analysis set.

17.3.2 PK analysis set (Part 2 only)

The PK analysis set (PKAS) will consist of all subjects who used at least 1 IP and provided at least 1 evaluable PK profile, and no major deviation or AEs judged to compromise the PK analysis. Individual PK values and/or full profiles for explicit IPs may be excluded from the analysis as specified in the SAP.

17.4 Pharmacokinetic analysis - general

The PK analysis will be based on the PKAS and performed by CTC. The PK parameters will be calculated by non-compartmental PK analysis using the software Phoenix WinNonlin® version 8.3 or later (Certara Inc., Princeton, NJ, US). In addition to $AUC_{0\text{-inf}}$ and C_{max} parameters, other non-compartmental PK parameters will be determined in relation to secondary endpoints (see Section 17.7.1 below). Where possible, both baseline-adjusted and non-adjusted PK parameters will be calculated.

For $AUC_{0\text{-inf}}$, the area under the plasma concentration *vs*. time curve will be calculated to the time point of the last quantifiable plasma concentration of nicotine and then extrapolated to infinity using the concentration in the last quantifiable sample and the estimated terminal elimination rate constant ($Lambda_z$).

PK data will be presented for each IP using summary statistics. This data will be presented in terms of N, arithmetic mean, median, SD, minimum and maximum value. For applicable PK parameters, the geometric mean and CV % will be presented. Categorical data will be presented as counts and percentages, as applicable.

17.5 Description of study population

17.5.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight, height, BMI, history of oral tobacco/nicotine use, Fagerström nicotine dependency test scores, and smoking will be presented for all subjects. All data will be listed by subject number.

17.5.2 Medical/surgical history and prior/concomitant medication

Medical/surgical history will be presented by system organ class (SOC) and preferred term (PT). Prior/concomitant medications will be presented by ATC level 4 and 5. All data will be listed by subject number.

17.5.3 Investigational product use

The number of subjects who used each IP will be presented through listings. Subject's own brand will be listed.

17.5.4 Physical examination

Physical examination parameters will be specified as "normal", "abnormal, not clinically significant", or "abnormal, clinically significant (as judged by the Investigator at the screening visit) and summarized.

All data will be listed by subject number.

17.5.5 Vital signs

Vital signs (systolic/diastolic blood pressure, and pulse rate) will be summarized by IP. All data will be listed by subject number.

17.5.6 Electrocardiogram

All ECGs will be categorized as" normal"," abnormal, not clinically significant", or" abnormal, clinically significant" (as judged by the Investigator at the screening visit) and summarized.

All data will be listed by subject number.

17.6 Analysis of primary endpoint

17.6.1 Comparison of the NP 11 mg and Swedish snus comparator ($AUC_{0\text{-}inf}$)

The primary endpoint of this study is to compare nicotine exposure, as measured by baseline-adjusted $AUC_{0\text{-}inf}$ based on nicotine plasma concentrations, between the NP 11 mg product and the comparator product, Swedish snus pouch 18 mg. The goal is to demonstrate that the upper bound of the 95 % confidence interval of the ratio for nicotine exposure of the moist NP 11 mg product and the comparator Swedish snus product is at or below 1.25.

$AUC_{0\text{-}inf}$ will be calculated as described in Section 17.4 above.

The comparison of $AUC_{0\text{-}inf}$ between the NP 11 mg and the comparator Swedish snus pouch 18 mg will be assessed using the following null (H_0) and alternative (H_1) hypotheses:

$$H_0: \frac{\mu_T}{\mu_R} > 1.25$$

$$H_1: \frac{\mu_T}{\mu_R} \leq 1.25$$

where μ_T is the least-squares geometric mean of $AUC_{0\text{-}inf}$ for the NP 11 mg and where μ_R is the least-squares geometric mean of $AUC_{0\text{-}inf}$ for the comparator Swedish snus pouch 18 mg.

In the analysis, a mixed model will be used with the natural log of $AUC_{0\text{-}inf}$ as the dependent variable, treatment as a fixed effect and subject as a random effect. The improved Kenward-Roger's approximation for degrees of freedom [23] will be used. The estimated least-squares means difference between the NP 11 mg and the comparator Swedish snus pouch 18 mg will be back-transformed into the original scale to present the ratio of geometric least square means as well as the corresponding 95 % confidence interval. If the upper bound of the interval of this ratio is at or below 1.25, the null hypothesis will be rejected, and it will be concluded that the NP 11 mg product does not result in substantially higher nicotine exposure compared to the comparator product.

17.7 Analysis of secondary endpoints

17.7.1 In vivo extracted amount and fraction of nicotine (Part 2 only)

The difference between the nicotine content of an unused reference pouch or loose snus portion and the used study pouch/portion will be used to calculate the *in vivo* extracted amount and fraction of nicotine for each IP. Extracted amount and fraction of nicotine will be presented using summary statistics. The amount of nicotine in the reference pouches or loose snus portions and in used pouches or loose snus portions will be presented through descriptive statistics.

In addition, extracted amount and fraction of nicotine will be analyzed exploratively using the same model as described in Section 17.6.1 to quantify estimated differences between products.

17.7.2 Pharmacokinetics of nicotine in plasma (Part 2 only)

The following non-compartmental PK parameters will be determined for each IP: $AUC_{0\text{-inf}}$, $AUC_{0\text{-last}}$, $AUC_{0\text{-1.5h}}$, C_{max}, T_{max}, and $T_{1/2}$. Where possible, both baseline-adjusted and non-adjusted PK parameters will be calculated.

C_{max} and T_{max} will be derived from the observed nicotine plasma concentration data. $AUC_{0\text{-1.5h}}$, $AUC_{0\text{-last}}$, and $AUC_{0\text{-inf}}$ will be calculated using log-linear trapezoidal interpolation. Calculations will be based on the actual sampling times recorded during the study. Concentrations below the lower limit of quantification (LLOQ) occurring before C_{max} will be treated as zero. Concentrations below LLOQ occurring after C_{max} will be omitted from the analysis. All baseline-adjusted PK parameters will be corrected for nicotine plasma concentrations at baseline (pre-use).

$AUC_{0\text{-inf}}$ will be calculated as described in Section 17.4 above.

The continuous secondary PK parameters (*i.e.*, all of the above except T_{max}) will be analyzed for both non-baseline-adjusted, and baseline-adjusted concentrations using the same model as described in Section 17.6.1 to assess non-inferiority between each test product and each comparator product respectively.

In addition, descriptive statistics for secondary PK parameters and concentrations (baseline-adjusted and non-baseline-adjusted) will be presented. PK plasma concentration curves over time will be presented.

All data will be listed by subject number.

17.7.3 Pharmacodynamic effects (Part 2 only)

Oximeter pulse rates will be presented in descriptive summary tables and in mean plots over time for each IP.

The subjective parameters “craving”, “satisfaction”, “product-liking” and “intent to use again”, measured by VAS, will be summarized for each IP using descriptive statistics during the IP use phase. Additionally, the relative and absolute change from baseline will be calculated for the subjective parameter “craving” for each IP.

To determine the maximum PD effect attributable to the IP, the highest recorded increase from baseline (E_{max}), time to first instance of E_{max} (T_{Emax}), the E_{max} from time 0 to 60 minutes ($E_{max0\text{-}60}$), and the time to reach $E_{max0\text{-}60}$ ($T_{Emax0\text{-}60}$) will be calculated for the pulse rates and presented using descriptive statistics.

For the subjective parameter "craving", the largest recorded decrease (E_{max}) will be calculated and presented using descriptive statistics.

For the subjective parameter "satisfaction", which does not have a baseline, the highest recorded value (E_{max}) and time to E_{max} will be calculated and presented using descriptive statistics.

17.7.4 Adverse events

An overview of all AEs, including SAEs, intensity, and deaths will be presented by study part and IP. The incidence of AEs and SAEs will be summarized by SOC and PT for each study part and IP.

All AE data will be listed by subject number and include the verbatim term entered by the Investigator.

17.8 Analysis of exploratory objectives

17.8.1 Exploratory subjective parameter (Part 2 only)

The subjective parameter "product-liking" *vs*. the subjects' usual snus/nicotine product of choice, measured through MCQ, will be summarized for each IP using descriptive statistics in a frequency table.

Additionally, the subgroup "loose snus users" will be analyzed and presented separately.

17.8.2 Extraction normalized $AUC_{0\text{-}inf}$ and C_{max} (Part 2 only)

Nicotine extraction normalized PK parameters $AUC_{0\text{-}inf}$ and C_{max} (the PK parameter with and without baseline-adjustment, divided by the extracted amount) will be determined for the following products: NP 6 mg, NP 11 mg, NP 16.6 mg, as well as for the comparators: own brand snus pouch/loose snus and Swedish snus pouch 18 mg. The analysis will compare the differences in $AUC_{0\text{-}inf}$, C_{max}, and the extracted amount and fraction of nicotine between the NP 6 mg, NP 11 mg, and NP 16.6 mg products, as well as for the comparators (own brand pouch/loose snus and Swedish snus pouch 18 mg). This analysis will use the same model described in Section 17.6.1.

Additionally, extraction normalized $AUC_{0\text{-}inf}$ and C_{max} data will be presented for each IP using summary statistics.

Additionally, the subgroup "loose snus users" will be analyzed and presented separately.

17.8.3 Ad libitum pattern of use (Part 1 and Part 3)

The pattern of use during the 14-day *ad libitum* usage periods will be summarized descriptively. This summary will include information on the total number of pouches/loose snus used per day, the estimated usage time per pouch/loose snus, and whether the subjects opened a new can each day. Additionally, the pattern of use data will be listed for both the own brand product period (Part 1) and the selected NP product during Part 3.

Additionally, the subgroup "loose snus users" will be analyzed and presented separately.

17.8.4 Exploratory pharmacokinetics of nicotine in plasma

The nicotine exposure, as measured by baseline-adjusted $AUC_{0\text{-}inf}$ and C_{max} based on nicotine plasma concentrations, will be compared between the selected strength NP product and the usual brand snus product, refer to Section 17.7.1.

Additionally, the subgroup “loose snus users” will be analyzed and presented separately.

17.8.5 Biomarker of exposure in urine (Part 1 and Part 3)

The difference in creatinine-normalized urine concentrations of the different BoE (nicotine and its metabolites, NNN, NNAL, and anabasine) of before and after complete substitution of own brand snus product with a moist NP product for 14 days will be analyzed using the same model as described under Section 17.6.1. The comparison of different BoE (nicotine and its metabolites, NNN, NNAL and anabasine, respectively) between each selected NP in Part 3 vs the subject’s own brand in Part 1 will be assessed using the following null (H_0) and alternative (H_1) hypotheses:

$$H_0: \frac{\mu_T}{\mu_R} < 0.8 \; or \; \frac{\mu_T}{\mu_R} > 1.25$$

$$H_1: 0.8 \leq \frac{\mu_T}{\mu_R} \leq 1.25$$

If the 90% confidence interval for geometric least square ratio falls entirely within the boundaries stipulated by H_1, equivalence between Part 1 and Part 3 in terms of BoE can be declared, lending credence to the idea of self-titration of nicotine exposure when substituting from an own brand to an NP product.

Additionally, the subgroup “loose snus users” will be analyzed and presented separately.

17.8.6 Nicotine, NNN and NNK extraction comparison (Part 1 and Part 3)

The difference in contents between unused and used pouches/loose snus portions will be used to calculate the *in vivo* extraction. The calculated extracted amounts of nicotine, NNK, and NNN per pouch/loose snus will be averaged per subject and multiplied with the consumption (number of pouches/loose snus used per day on average) reported in the eDiary over the 14 days *ad libitum* usage period, to receive the daily exposure.

Daily exposure to nicotine, NNK, and NNN will be summarized descriptively and analyzed using the same model and principles as outlined under Section 17.8.5.

Average extracted amounts (mg/unit) and fractions (%) of nicotine, NNK, and NNN will also be summarized descriptively.

Additionally, the subgroup “loose snus users” will be analyzed and presented separately.

17.8.7 Nicotine, NNN and NNK extraction correlation to biomarkers of exposure (Part 1 and Part 3)

Daily exposure to nicotine, NNK, and NNN will be calculated as described under Section 17.8.6. The intake will be correlated with urine concentrations of BoE (nicotine, cotinine, NNN, NNAL, and anabasine) for dose-relationship investigations using linear regression with BoE levels as the dependent variable and daily exposure to nicotine, NNK, and NNN as a continuous explanatory variable in separate models. Subject will be included in the models as a random effect.

In total the following models will be developed (refer to Table 17.8-1):

Table 17.8-1 Correlation analysis models

Model number:	**Dependent variable:**	**Explanatory variable:**
Model 1	Nicotine (Nic) in urine	Daily exposure to nicotine
Model 2	Cotinine (Cot) in urine	Daily exposure to nicotine
Model 3	4-(Methylnitrosamino) -1-(3-pyridyl) -1-butanol (NNAL) in urine	Daily exposure to nicotine
Model 4	*N*-nitrosonornicotine (NNN) in urine	Daily exposure to nicotine
Model 5	Anabasine in urine	Daily exposure to nicotine
Model 6	3'-trans-hydroxycotinine (OH-Cot) in urine	Daily exposure to nicotine
Model 7	Nicotine equivalents in urine	Daily exposure to nicotine
Model 8	4-(Methylnitrosamino) -1-(3-pyridyl) -1-butanol (NNAL) in urine	Daily exposure to NNK
Model 9	*N*-nitrosonornicotine (NNN) in urine	Daily exposure to NNN

To all of the models above, the product (including subject's own brand, NP 6 mg, NP 11 mg, and NP 16.6 mg) will be fitted as dummy variables. Additionally, the interaction effect between daily exposure to nicotine/NNK/NNN and the product will be considered, resulting in different regression slopes for each product. The regression results will be presented graphically, along with model fit statistics and p-values from hypothesis tests, highlighting differences between products in terms of daily exposure to nicotine/NNK/NNN and the BoE correlation. Depending on model diagnostics, the analysis approach may be updated – for instance, grouping NP products or using scatter plots to visualize relationships without inferential statistics.

Additionally, the subgroup "loose snus users" will be analyzed and presented separately.

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19 SIGNATURES

19.1 Approval of the clinical study protocol

I, the undersigned, approve this CSP.

Sponsor signatory

[redacted]

Coordinating Investigator

[redacted]

19.2 Principal Investigator statement

I, the undersigned, have read and understood this CSP and agree to conduct the study accordingly, complying with the Investigator obligations stated in this CSP, GCP, and applicable regulatory requirements.

_____

Name, title

_____

Site