Clinical Study Protocol	
EudraCT No.	NA
Investigational Product	ZYN®
Study Code	SM 18-01
Protocol Version and Date	Final 3.0, 16 Jan 2019

Nicotine plasma concentrations and pharmacokinetics of single doses of non-tobacco-based nicotine pouches (ZYN®) compared with conventional, tobacco-based Swedish snus and American moist snuff among current, daily snus users.

Phase	NA
Design	Open, randomized, seven-way cross-over, single dose administration.
Indication	NA
Test product and dosage	**1**= ZYN Smooth containing 6 mg nicotine per portion **2**= ZYN Smooth containing 8 mg nicotine per portion **3**= ZYN Wintergreen containing 6 mg nicotine per portion **4**= ZYN Smooth containing 6 mg nicotine per portion (lower lip)
Comparator product and dosage	**5**= General PSWL (8 mg nicotine/g) 2 x 1.0 g **6**= Longhorn Pouch Natural (12 mg nicotine/g) 1.5 g **7**= Longhorn Pouch Wintergreen (12 mg nicotine/g) 1.5 g
Duration of treatment	Approximately 7 weeks
Sponsor signatory	[redacted] Swedish Match SE-Box 17037 104 62 Stockholm Sweden Maria Skolgata 83 SE-118 85 Stockholm Sweden [redacted]
Principal Investigator	Erik Rein-Hedin, MD

	CTC Clinical Trial Consultants AB
Clinical study conduct and management	CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden CTC Clinical Trial Consultants AB Uppsala University Hospital, Entrance. 85, 2nd level SE-751 85 Uppsala, Sweden

1 STUDY SYNOPSIS

Study Title

Nicotine plasma concentrations and pharmacokinetics of single doses of non-tobacco-based nicotine pouches (ZYN®) compared with conventional, tobacco-based Swedish snus and American moist snuff among current, daily snus users.

Study code	**EudraCT No**
SM 18-01	*NA*
Study period Estimated date of first subject enrolled: *Q4-2018* Estimated date of last subject completed: *Q1-2019*	**Phase of development** *NA*

Coordinating Investigator

Erik Rein-Hedin, MD, CTC Clinical Trial Consultants AB

Study design

Open, randomized, seven-way cross-over, single dose administration in healthy volunteers.

Objectives

Primary objective

The primary objective(s) of the study is to evaluate each subject's plasma concentrations of nicotine after administration of one single dose of ZYN® Smooth containing 6 mg of nicotine, to that of one single dose of 2x1 pouches of General PSWL Swedish snus.

Secondary objectives

- To compare Tmax, Cmax, AUCinf, AUC0-t from each dose of the non-tobacco-based nicotine ZYN® Smooth pouches, General PSWL Swedish snus pouches and Longhorn American moist snuff pouches.

-To assess the effect of the flavour component (Methyl salicylate) on nicotine plasma concentrations for the non-tobacco-based nicotine pouch ZYN® Wintergreen and Longhorn Wintergreen pouch American moist snuff, respectively.

- To assess if there is a difference in nicotine plasma concentrations between upper lip and lower lip placement of the non-tobacco-based nicotine pouch ZYN® Smooth.

- To compare the estimated in-vivo extracted amount and rate of extraction of nicotine from each dose.

- To assess plasma levels of Methyl salicylate for the treatments with pouches containing Wintergreen flavour.

- To compare Adverse events from each dose.

Endpoints

Primary endpoints

AUCinf based on plasma concentrations of nicotine after administration of a single dose of ZYN® Smooth containing 6 mg of nicotine, to that of two doses of General PSWL Swedish snus pouches.

Secondary endpoints

- Tmax, Cmax, AUCinf, AUC0-t and terminal half-life of the non-tobacco-based nicotine ZYN® Smooth pouches, General PSWL Swedish snus pouches and Longhorn American moist snuff pouches.

- Nicotine plasma concentrations for the ZYN® Wintergreen and Longhorn Wintergreen treatments compared to the corresponding non-wintergreen containing product, respectively.

- Nicotine plasma concentrations for the upper lip and lower lip placement of the non-tobacco-based nicotine pouch ZYN® Smooth.

- In-vivo extracted amount of nicotine from all products.

- Pairwise analysis of in-vitro extracted nicotine and rate of extraction.

- To analyze plasma levels of Methyl salicylate for the treatments with pouches containing Wintergreen flavour.

- Collection and comparison of adverse events

Number of subjects/patients planned

A maximum of 36 subjects will be randomized in the study so that approximately 32 evaluable subjects complete the study.

Main eligibility criteria

For inclusion in the study, subjects/patients must fulfil the following criteria:

1. Snus user who have used snus for ≥1 year, with a minimum weekly consumption of two or more snus cans (preferably brands with nicotine content ≥1%).

2. Willing and able to give written informed consent for participation in the study.

3. Healthy male or female subject aged ≥19 years of age inclusive.

4. Willing and able to comply with study procedures.

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

1. Smoker, defined as "smoking during the last 24 hours according to self-report and CO in exhaled air >10 ppm at clinical visits.

2. A history or presence of diagnosed hypertension or any cardiovascular disease.

3. Surgery within 6 months of the screening visit that, in the opinion of the investigator, could negatively impact on the subject’s participation in the clinical study.

4. Any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the investigational product.

5. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject’s ability to participate in the study.

6. Breast feeding, pregnancy or planning to get pregnant during the study.

7. Female use of systemic contraceptives (such as oral contraceptives, implants, injectable steroids, vaginal ring, transdermal patch).

8. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).

9. Positive screen for drugs of abuse or alcohol at screening or on admission to the unit prior to administration of the IP.

10. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse.

11. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the three months prior to screening.

12. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.

Methodology

A crossover design was chosen to yield a more efficient comparison of treatments than a parallel study design, i.e., fewer subjects are required since each subject will serve as his own control. To avoid carryover effects, a wash-out period of at least 24 hours has been incorporated between administrations.
Investigational Products (IP), dosage and mode of administration The test products of ZYN® Smooth contains 6 and 8 mg of nicotine, respectively, in each pouch while ZYN® Wintergreen contains 6 mg of nicotine in each pouch. The reference product with Swedish snus, 2 x 1 g General PSWL pouch, contains 8 mg of nicotine/g while the reference products with American moist snuff, 1.5 g Longhorn Natural Pouch and Longhorn Wintergreen Pouch, contains 12 mg nicotine/g. All investigational products will be in the form of pouches administered at the CTC clinic.
Duration of treatment During the cross-over phase of the study, visit 2 to 8, the participating subjects will be given the study products for a period of 60 minutes on each visit.
Duration of subject's involvement in the study Each subject will participate in the study for a period of approximately 7 weeks.
Pharmacokinetic (PK) assessments The pharmacokinetic assessments in the study will include AUCinf, AUC0-t, Cmax, Tmax, and terminal half-life.
Nicotine extraction assessment Amount and rate of extraction of nicotine will be evaluated for all products.
Flavour component (Methyl salicylate) assessment Plasma levels of the flavor component methyl salicylate will assessed for the products ZYN® Wintergreen and Longhorn Wintergreen pouch.
Statistical methods Pharmacokinetic data will be presented using summary statistics. Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), median, minimum and maximum value. In addition, for the parameters AUC and Cmax 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 treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time. The primary endpoint will be investigating a one sided hypothesis that ZYN 6mg has a lower AUC compared to 2 pouches of 8 mg Swedish snus, using a significance level of 2.5%. All other test will be two-sided with a significance level of 5%.
Study reporting After completion of the study, an ICH-E3 compliant clinical study report (CSR) will be prepared.

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or term	Explanation
ADL	Activities of daily living
AE	Adverse event
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma concentration time curve
AUC_{inf}	Area under the curve from 0 to infinity
AUC_{last}	Area under the curve from 0 to t hours where t is the last measured concentration
C_{max}	Maximum (peak) concentration
CO	Carbon monoxide
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
eCRF	Electronic case report form
EEA	European Economic Area
FIH	First-in-human
FDA	U.S. Food and Drug Administration
GCP	Good clinical practice
Hb	Haemoglobin
HIV	Human immunodeficiency virus
h	hour
ICF	Informed consent form
ICH	International conference on harmonization
IEC	Independent ethics committee
IP	Investigational product
LLOQ	Lower limit of quantification
MedDRA	Medical dictionary for regulatory activities
min	minute
N	number
NCA	Non-compartmental analysis
NIH	National Institute of Health

OTC	Over the counter
PK	Pharmacokinetic
PPAS	Per protocol analysis set
PSWL	Pouched Snus White portion Large
PT	Preferred term
RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SDV	Source data verification
sec	Second
SOC	System organ class
SOP	Standard operating procedures
TEAE	Treatment emergent adverse event
T_{max}	Time after drug administration when the maximum plasma concentration is reached
$T_{½}$	Half life

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical emergencies contacts

The Principal Investigator 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 included in Section 10.5.7.

In the case of a medical emergency the Investigator may contact the Medical Responsible Person at Swedish Match.

[redacted]

5 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor
Swedish Match
SE-Box 17037
104 62 Stockholm,
Sweden
Maria Skolgata 83, 1 tr
SE-11885 Stockholm, Sweden

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Clinical conduct
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Study management
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Signatures required are provided in Appendix 17.1.

6 INTRODUCTION

6.1 Background

Sweden has developed the lowest prevalence of smoking in Europe, particularly among males. It is widely accepted that one contributory factor to this trend is that snus has replaced cigarettes as the tobacco product of choice among many male and some female smokers.

Oral tobacco, like snus, is capable of rapidly delivering nicotine to the venous bloodstream through diffusion over the oral mucosa (Fant et al 1999) [1]. It may therefore be more satisfactory to smokers than currently available pharmaceutical nicotine replacement therapies (NRT). However, orally administered nicotine, whether in the form of snus or NRT, can never achieve the very high nicotine levels in the arterial blood to the brain that is typically associated with pulmonary delivery resulting from inhalation of tobacco smoke.

Use of smokeless tobacco is by definition unassociated with exposure to the many thousands of combustion products 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 smokeless tobacco products has substantially lower health risks than cigarette smoking. However, smokeless tobacco typically entails a systemic exposure to nicotine that is comparable to that among cigarette smokers.

Nicotine is the substance that is thought to contribute the most to the addictive properties of using any type of tobacco product. Also, the nicotine exposure may help to explain the adverse pregnancy outcomes that have been observed among pregnant women who continue to use tobacco after the first trimester of their pregnancy. Such effects have been documented both for cigarette smoking and use of snus. In addition, smokeless tobacco products typically contain low levels of unwanted substances (including nitrosamines and polycyclic hydrocarbons) that have been classified as human carcinogens. So, although the health effects are substantially smaller for smokeless tobacco than for cigarette smoking, some adverse effects cannot be ruled out, in particular not effects related to the nicotine exposure.

Traditionally there has been no non-tobacco-based nicotine product on the Swedish market intended for recreational use. Despite the vast risk differential between snus and cigarettes in terms of adverse long-term health effects, snus remains a controversial product as it contains tobacco, is intended for recreational use, and is potentially addictive. The tobacco component of snus explains why it contains measurable amounts of unwanted, potentially carcinogenic constituents, albeit at very low concentrations.

Recently, a novel, non-tobacco-based nicotine product (ZYN®) has been developed and is now commercially available both in Sweden and in the U.S. It has some features that are similar to snus. It comes in pouches that are intended to be placed under the upper lip (although some consumers, particularly in the U.S., place the product under the lower lip). In contrast to snus, the product contains no nitrosamines or polycyclic hydrocarbons which are the two main classes of unwanted substances in snus. In ZYN® products, the matrix used for nicotine delivery consists of microcrystalline cellulose and maltitol, two inert substances frequently used in food stuffs. ZYN® does contain some unwanted substances but they are present in concentrations comparable to or lower than in many commonly used food stuffs or snus. The toxicological safety profile of ZYN® thus represents a significant improvement over snus. However, the nicotine content is comparable to that in snus and many other smokeless tobacco products that are currently common on the market in Scandinavia and the U.S.

When comparing the nicotine content of different nicotine-delivery products it is important to consider that the nicotine uptake varies considerably depending on product type (tobacco versus a non-tobacco-based matrix) and product formulation (pouch geometry, water content, particle size, pH, etc). In addition, there is a substantial inter-individual variation in uptake with products used orally. This is probably related to constitutional differences in saliva production and a resulting wide variation in nicotine extraction from the product. It has also been suggested that some commonly used additives may affect nicotine uptake through an effect on saliva surface tension. For instance, the flavouring agent methyl salicylate ("wintergreen") has been hypothesized to facilitate oral nicotine uptake through a decreased saliva surface tension.

The placement of the pouch under the upper versus the lower lip may theoretically also affect nicotine extraction and hence uptake. There are to date no data on whether pouch placement is a relevant factor but, theoretically, placement under the upper lip implies that the pouch is located at a distance from the orifices of large salivary glands. On the other hand, placement under the lower lip implies that the pouch may be more soaked in saliva (which is typically more abundant in the lower part of the mouth) and hence that nicotine extraction may be more rapid and/or complete.

Commercially available snus products have a nicotine content ranging between 1-2%. Previous studies (Lunell E and Curvall M 2011)[2], have indicated that on average about 15-20% of the total nicotine content is extracted and absorbed although with large inter-individual variation. Extraction is generally not linear with pouch size: it is larger with small compared to larger pouches, which suggests that surface area, saliva penetration and diffusion factors may be more important determinants of nicotine uptake than pouch weight.

The nicotine delivery profile of a product is probably one main determinant of its efficacy to decrease nicotine craving and, thus, its ability to function as an alternative to cigarettes among current smokers. At the same time, it probably also helps to explain the product's addictive properties.

A previous in vivo nicotine extraction study compared the ZYN® 3 and ZYN® 6 mg products to a conventional snus product (1.0-gram pouch) with 8 mg of nicotine. Nicotine extraction was measured after 15 and 60 minutes. The study showed that the 3 mg ZYN® product was associated with a nicotine extraction that was less compared to the 8 mg snus product, whereas slightly more nicotine was extracted from the 6 mg ZYN® product. As extraction probably is a proxy for nicotine uptake, it is reasonable to assume that also the uptake is slightly higher with the 6 mg product. This hypothesis is currently being tested in a formal nicotine uptake study. A limitation of the mentioned studies was that they did not include the newly developed ZYN® 8 mg product. That product was not commercially available at the time of study initiation.

When comparing nicotine uptake and exposure from currently available nicotine delivery products it is important to consider that many snus brands have a nicotine content that is higher than the 8 mg/pouch product used in the mentioned in vivo extraction study. Also, according to a consumer survey 10-15% of all snus users frequently use two or more pouches simultaneously. Users of loose snus typically use pinches weighing 2-2.5 grams. So, the mentioned results with a 1.0-gram pouch may underestimate and not adequately reflect the level of nicotine exposure experienced by current snus users.

In view of these circumstances, it is justified to study the nicotine delivery and uptake profile of the ZYN® (6 and 8 mg) products in comparison with some commercially available snus products on the Scandinavian and U.S. markets (see Table 6.1-1). These brands typically have a higher nicotine content and/or larger pouch size than the comparator snus product used in the mentioned in vivo extraction study. Given that 10-15% of snus users often use two or more pouches simultaneously, it is reasonable to study the nicotine uptake with such an exposure. Also, it is motivated to assess whether the commonly used flavouring compound methyl salicylate ("wintergreen") may affect nicotine uptake, and to address the putative effect of pouch placement (upper versus lower lip).

Table 6.1-1 Product characteristics

Test article	Pouch size (g)	Nicotine content (mg/g)	Nicotine content (mg per unit)	pH
ZYN Smooth 6 mg	0.4	15	6	8.3
ZYN Wintergreen 6 mg	0.4	15	6	8.3
ZYN Smooth (lower lip) 6 mg	0.4	15	6	8.3
ZYN Smooth 8 mg	0.5	15	8	8.3
General PSWL (2 x 1.0 g)	1.0	8	16 (2 units)	8.7
Long Horn Pouch Natural	1.5	12	18	8.0
Long Horn Pouch Wintergreen	1.5	12	18	8.0

Note: The cited product characteristics represent production target values. This implies that there can be some batch to batch variation.

6.1.1 *Product characteristics*

Test and reference products will be delivered in identical containers labeled with unique identification numbers.
The test products of ZYN® Smooth contains 6 and 8 mg of nicotine, respectively, in each pouch while ZYN® Wintergreen contains 6 mg of nicotine in each pouch.
The reference product with Swedish snus, 2 x 1 g General PSWL pouch, contains 8 mg of nicotine/g while the reference products with American moist snuff, 1.5 g Longhorn Natural Pouch and Longhorn Wintergreen Pouch, contains 12 mg nicotine/g.
Administration of the pouch will be between the upper lip and the gum except for one treatment when the pouch is placed between the lower lip and the gum.

6.2 Study rationale

The overarching aim of the study is to ensure that the ZYN® products do not entail a higher nicotine exposure than is the case with commercially available tobacco-based snus or snus-like products that are currently common on the Scandinavian and U.S. markets.

6.3 Risk/benefit assessment

It may be considered problematic to expose research subjects to a novel nicotine delivery product, the properties of which are not yet fully known. However, all research subjects are required to be daily snus users since at least one year (with an average or above snus consumption) so the participants are well acquainted with, and used to, the effects of nicotine.

Preliminary data from previous studies indicate that the amount of nicotine extracted from the test articles is comparable to that from tobacco-based snus, despite the fact that the overall nicotine content and content of free nicotine in the ZYN® pouches is lower than in conventional tobacco-based snus (8 mg). This suggests that adverse effects from the nicotine exposure from the test and reference articles are unlikely to occur among the research subjects. So far, no adverse effects have been reported associated with the use of ZYN® apart from effects likely to be related to the nicotine exposure (such as salivation, nausea, and dyspepsia).

Aside from the nicotine, all ingredients used in the test products are food-approved (similar to ingredients in conventional snus). The nicotine in ZYN® is of pharmaceutical grade, i.e. the same as the nicotine in nicotine replacement products (gum, lozenges, mouth spray etc.). ZYN® is currently commercially available on the U.S. and Swedish markets.

The study does not involve invasive procedures, beside the collection of venous blood samples.

Pregnant women or individuals with a history of hypertension or any cardiovascular disease, who may be particularly vulnerable to nicotine exposure, are excluded from participation.

The potential adverse effects of the study procedures, which are likely to be minor and/or clinically insignificant, are from a research ethics perspective counterbalanced by the potential positive effects of the novel nicotine pouch as a reduced toxicity alternative to conventional snus. As the nicotine delivery profile of a product is likely to be central to its acceptability among current tobacco users, it is reasonable to conduct formal clinical studies to assess this feature in more detail.

Subjects will remain in the research clinic for at least 6 hours after the administration of the investigational products and will be closely monitored by medical staff.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objective(s)

The primary objective(s) of the study is to evaluate each subject's plasma concentrations of nicotine after administration of one single dose of ZYN® Smooth containing 6 mg of nicotine, to that of one single dose of 2x1 pouches of General PSWL Swedish snus.

7.1.1 *Primary endpoint(s)*

AUC_{inf} based on plasma concentrations of nicotine after administration of a single dose of ZYN® Smooth containing 6 mg of nicotine, to that of two doses of General PSWL Swedish snus pouches.

7.2 Secondary objectives

The secondary objectives of the study are to evaluate pharmacokinetic variables for the remining products, plasma levels of Methyl salicylate and local tolerability.

1. To compare T_{max}, C_{max}, AUC_{inf}, AUC_{0-t} from each dose of the non-tobacco-based nicotine ZYN® Smooth pouches, General PSWL Swedish snus pouches and Longhorn American moist snuff pouches.

2. To assess the effect of the flavour component (Methyl salicylate) on nicotine plasma concentrations for the non-tobacco-based nicotine pouch ZYN® Wintergreen and Longhorn Wintergreen pouch American moist snuff, respectively.
3. To assess if there is a difference in nicotine plasma concentrations between upper lip and lower lip placement of the non-tobacco-based nicotine pouch ZYN® Smooth.
4. To compare the estimated *in-vivo* extracted amount and rate of extraction of nicotine from each dose.
5. To assess plasma levels of Methyl salicylate for the treatments with pouches containing Wintergreen flavour.
6. To compare Adverse events from each dose.

7.2.1 ***Secondary endpoints***

1. T_{max}, C_{max}, AUC_{inf}, AUC_{0-t} and terminal half-life of the non-tobacco-based nicotine ZYN® Smooth pouches, General PSWL Swedish snus pouches and Longhorn American moist snuff pouches.
2. Nicotine plasma concentrations for the ZYN® Wintergreen and Longhorn Wintergreen treatments compared to the corresponding non-wintergreen containing product, respectively.
3. Nicotine plasma concentrations for the upper lip and lower lip placement of the non-tobacco-based nicotine pouch ZYN® Smooth.
4. *In-vivo* extracted amount of nicotine from all products.
5. Pairwise analysis of in-vitro extracted nicotine and rate of extraction.
6. To analyze plasma levels of Methyl salicylate for the treatments with pouches containing Wintergreen flavour.
7. Collection and comparison of adverse events

8 STUDY DESIGN

8.1 Overall study design and schedule of events

The study will be conducted as an open, randomized, seven-way cross-over, single dose administration. The study will include 36 subjects.

The subjects included will be healthy males and females aged ≥19 years who have used tobacco-based snus for ≥1 year with a weekly consumption of three or more snus cans (brands with nicotine content ≥1%). Subjects who are pregnant or who have a history of hypertension or any cardiovascular disease are excluded. Subjects shall be abstinent from snus and all other nicotine containing products from 8.00 p.m. the night before each trial day.

Before entry to the study subjects undergo screening, evaluations including smoking and snus use, medical history and collection of height and weight.

Subjects report to the clinic on separate days for the seven (7) experimental sessions in addition to visits for screening and follow-up. Between each experimental session a wash-out period of at least 24 hours is required. The subjects are instructed to abstain from snus or other nicotine delivery products as from 8.00 p.m. the evening before and to refrain from smoking 24 hours before each experimental session. All sessions are performed during the morning hours to facilitate abstinence. The subjects should certify abstinence before each treatment is started.

The treatments are administered as single doses in a pre-determined randomized order. The subject keeps the pouch still between the upper lip (lower lip for treatment 4) and the gum for 60 minutes and are instructed not to manipulate the pouch with the tongue or lips. The subjects are instructed not to eat, drink, chew chewing gum or brush teeth from 30 minutes before application of treatment, during application of investigational products and 30 minutes after the investigational product have been taken out (Henningfield et al 1990)[3].

After 60 minutes the pouches are collected and frozen (-20°C) pending analyses of nicotine.

Blood samples for assessment of plasma levels of nicotine and Methyl salicylate will be collected at pre-defined time points from pre-dose to 6 hours after IP administration.

Table 8.1-1 Schedule of events

Visit	**Visit 1[1] Screening**	**Visit 2-8[2] Cross-over**	**Visit 9 Telephone FU**
Assessments / Study days	*-28 to -1*		*7 days(-3/+7) after last dose*
Informed consent	x		
Demographics	x		
Medical/surgical history	x		
Inclusion/exclusion criteria	x		
Physical examination	x		
Weight, height	x		
HIV, Hepatitis B and C	x		
Pregnancy test[3]	x		
Drugs of abuse	x	x[4]	
Alcohol screen	x	x[4]	
CO measurement	x	x	
Randomisation		x[5]	
IP (pouch) administration		x	
PK blood sampling		x[6]	
Collection of pouches		x	
Treatment-emergent AEs (TEAEs)[8]		x	x
Baseline events	x	x[7]	
Prior and concomitant medications[9]	x	x	x

[1] Visit 1 could be performed during 2 days

[2] Refer to section 8.1.3, Schedule of Events per visit for, details.

[3] Female subjects only

[4] Drug and alcohol tests during the treatment period may be performed randomly.

[5] Only on visit 2

[6] Before and 5, 10, 15, 30, 60, 90, 120, 240 and 360 min after application of the investigational products

[7] Only prior to dose administration at Visit 2.

[8] From first administration of IP.

[9] For definitions of prior and concomitant medication, see Section 10.2.7.

Table 8.1-2 Detailed schedule of events for each treatment period

Visit	Visit 2 to 8											
Assessment/time-point	**Admission**	**Pre-dose**	**0**	**5 min**	**10 min**	**15 min**	**30 min**	**60 min**	**90 min**	**2 h**	**4 h**	**6h**
Inclusion/exclusion criteria	x[1]											
CO measurement	x											
Drugs of abuse	x[2]											
Randomisation		x[3]										
IP (pouch) administration			x									
IP (pouch) collection								x[4]				
PK blood sampling		x		x	x	x	x	x	x	x	x	x
Treatment-emergent AEs (TEAEs)			x									
Prior and concomitant medications	x											

[1] Confirmation of inclusion/exclusion criteria (Visit 2 only).

[2] Drug tests during the treatment period may be performed randomly

[3] Only on visit 2

[4] Collection of pouch +/- 1 (one) minute from specified time.

8.2 Rationale for study design

A crossover design was chosen to yield a more efficient comparison of treatments than a parallel study design, i.e., fewer subjects are required since each subject will serve as his own control. To avoid carryover effects, a wash-out period of at least 24 hours has been incorporated between administrations.

Randomisation will be used to minimise bias in the assignment of subjects to a treatment sequence and to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) are evenly balanced across treatment groups.

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

8.3 Recruitment

The subjects will be recruited from a list of healthy volunteers at CTC and from advertising in media.

8.4 Screening and enrolment 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 screen failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects included but not completed.

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

Subjects included and randomised will be assigned a randomisation number (101,102 and 103 etc.).

8.5 Number of subjects

36 subjects will be included in the study.

For replacements of subjects discontinuing the study, see Section 8.5.

8.6 Inclusion criteria

For inclusion in the study, subjects/patients must fulfil the following criteria:

1. Snus user who have used snus for ≥1 year, with a minimum weekly consumption of two or more snus cans (preferably brands with nicotine content ≥1%).
2. Willing and able to give written informed consent for participation in the study.
3. Healthy male or female subject aged ≥19 years of age inclusive.
4. Willing and able to comply with study procedures.

8.7 Exclusion criteria

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

1. Smoker, defined as "smoking during the last 24 hours according to self-report and CO in exhaled air >10 ppm at clinical visits.
2. A history or presence of diagnosed hypertension or any cardiovascular disease.
3. Surgery within 6 months of the screening visit that, in the opinion of the investigator, could negatively impact on the subject's participation in the clinical study.
4. Any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the investigational product.
5. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
6. Breast feeding, pregnancy or planning to get pregnant during the study.
7. Female use of systemic contraceptives (such as oral contraceptives, implants, injectable steroids, vaginal ring, transdermal patch).
8. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
9. Positive screen for drugs of abuse or alcohol at screening or on admission to the unit prior to administration of the IP.
10. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse.
11. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the three months prior to screening.
12. Investigator considers the subject unlikely to comply with study procedures, restrictions and requirements.

8.8 Restrictions during the study

The subjects must be willing to comply to the following restrictions during the entire study duration *i.e.*, from screening to the last Follow-up Visit.

8.8.1 *General restrictions*

- Subjects shall abstain from snus and all other nicotine containing products from 8.00 p.m. the night before each study day.
- Subjects shall abstain from smoking the last 24 hours before each study day.
- The subjects are not allowed to eat or drink or use any other mouth related procedure (e.g. tooth brushing) 30 minutes before dose administration, during application of investigational products and 30 minutes after the investigational product have been taken out. [3].
- The female volunteers are expected to be sexually abstinent or use non-systemic contraceptives to prevent pregnancy during the study period.

- Abstain from use of Methyl salicylate containing products, e.g. ointments, mouth wash and chewing gum.
- Abstain from drugs of abuse from Screening to the Follow-up visit.
- The subjects must not donate blood or plasma during the study until three months after the Follow-up Visit.
- Study subjects are not allowed to participate in any other clinical study during the study period i.e screening to follow up.

8.8.2 *Prior and concomitant therapy*

Female systemic contraceptives e.g. birth control pills, injectable steroids, vaginal ring, transdermal patch and contraceptive implants are not allowed in the study.

Other concomitant medications or therapies, including herbal remedies, vitamin supplements and over-the-counter (OTC) products, will be allowed during participation in the study.

8.9 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but are no subsequently randomised/entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects/patients to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements ad to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details and eligibility criteria.

Subjects/patients who do not meet the criteria for participation in this study may be re-screened.

8.10 Criteria for subject/patient withdrawal

8.10.1 *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 for any of the following reasons:

- Severe non-compliance to study protocol procedures, as judged by the Investigator and/or Sponsor
- Subject is lost to follow-up. A subject will be considered lost to follow-up if he/she fails to come for 2 number of scheduled visits and if he/she is not possible to contact by site staff despite several attempts.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor

8.10.2 *Procedures for discontinuation of a subject/patient from the study*

A subject/patient who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. If possible, they

will be seen by the Investigator and assessed according to the procedures scheduled for the follow-up visit/early termination. Any ongoing AEs will be followed as described in Section 10.5.8.

8.10.3 *Subject replacement*

Subjects who are prematurely withdrawn from the study may not be replaced during the course of the study.

8.11 Randomization

On study Day 1 (Visit 2), the subjects will be randomised to a treatment sequence. As this is an open study, the treatments to which each subject is allocated for the first dose administrations will be recorded in the eCRF. A computer-generated randomisation list will be created using SAS Proc Plan, SAS Version 9.4. The randomisation list will contain subject number, treatment sequence, period, and treatment.

The randomisation list will be generated by CTC or delegate and provided to the packing company. The original randomisation list will be kept by the randomiser. A copy of the randomisation list will be provided to the clinic. A coded copy of the randomisation list will be generated, i.e. a list where the treatment is blinded and this coded list will be provided to the bioanalytical laboratory and to the pharmacokineticist.

9 TREATMENTS

9.1 Identity of investigational products

The investigational products (IP), both test- and reference products are supplied by Swedish Match AB

9.1.1 *Test products*

1= ZYN® Smooth containing 6 mg nicotine per portion

2= ZYN® Smooth containing 8 mg nicotine per portion

3= ZYN® Wintergreen containing 6 mg nicotine per portion

4= ZYN® Smooth containing 6 mg nicotine per portion (lower lip)

9.1.2 *Reference products*

5= Swedish portion snus, General PSWL (8 mg nicotine/g) 2x1.0 g

6= American moist snuff, Longhorn Pouch Natural (12 mg nicotine/g) 1.5 g

7= American moist snuff, Longhorn Pouch Wintergreen (12 mg nicotine/g) 1.5 g

9.2 Packaging and labelling

The IP will be manufactured and packaged in compliance with the Swedish law on food production.
The IP will be transferred from the original container, weighed and individually packaged in identical sealed food approved test containers at the Swedish Match analytical lab.
The containers will be labeled with unique identification numbers by Swedish Match in accordance with the randomisation list.

9.3 Conditions for storage

The IP will be stored in an access-controlled storage area at CTC, as per storage conditions specified by the Sponsor.

Temperature logs will be kept for the area where the IP is stored. The temperature should be noted on a daily basis (working days only unless automatic temperature readings are available).

9.4 Dispensing and accountability

The IP will be dispensed as per the randomization schedule by site personnel.

CTC AB and the Investigator will maintain a Drug Dispensing Log detailing the dates and quantities of IP received, dispensed to and used by each subject as well as IP returned or destroyed at the end of the study. Any discrepancies between dispensed and returned IP must be explained and documented. Products deliberately and/or accidentally destroyed by the Investigator or the subject/patient must be accounted for.

9.5 Treatment administration

A single dose will be given on the morning of each study day.

9.6 Continuation of treatment with Investigational Product

In this study the healthy volunteers who participate will have no medical benefit from the treatment and thus there will be no treatment with investigational or reference products after end of study participation.

9.7 Treatment compliance

All IP will be administered at the research clinic under medical supervision to ensure compliance.

9.8 Return and destruction of investigational products

Any unused IP will be destroyed at the study site. Empty containers will be destroyed at the study site. The Monitor will perform final IP accountability reconciliation at the study end to verify that all unused IP is adequately destroyed/returned and documented.

10 STUDY ASSESSMENTS

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

10.1 Recording of data

The Principal Investigator will provide the Sponsor with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the eCRF and in all required reports.

It is important that PK blood sampling occurs as close as possible to scheduled time. In order to achieve this, the timing priority order at a particular time point is:

1. IP pouch collection
2. Blood samples for PK

The time points for PK blood sampling will start from the administration of the pouch.

The plasma sample may be drawn with some deviation from the time stated in the protocol. The time window for each time point see section 10.3.1.

The actual sampling time should always be recorded in the eCRF and will be used in the calculation of the PK parameters. Pre-dose assessments may be performed up to 60 minutes prior to dosing (if not otherwise specified in the schedule of events).

10.2 Demographics and other baseline characteristics

10.2.1 *Informed consent*

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 13.3.

10.2.2 *Eligibility criteria*

Eligibility criteria should be checked during screening and verified before randomisation/IP administration. The criteria are specified in Sections 8.6 and 8.7.

10.2.3 *Demographic information*

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

10.2.4 *Weight and height*

Weight and height will be measured without shoes. BMI will be calculated from the height and weight recorded and rounded off to the nearest whole number.

10.2.5 *Physical examination*

A complete physical examination will include assessments of general appearance, throat, thyroid, lungs, cardiac, abdomen (liver and spleen), lymph nodes and extremities.

10.2.6 *Medical/surgical history*

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

10.2.7 *Prior and concomitant medication*

Prior medication will be obtained by subject interview in order to verify that the eligibility criteria are met (see also Section 8.8.2).

Medications are classified as prior if the stop date was before or on the day of the first dose administration (pre-dose) and as concomitant if ongoing at the day of the first dose administration, stopped after the first dose administration or started after the first dose administration.

Any use of concomitant medication from screening until the last Follow-up Visit must be documented appropriately in the subject's eCRF. Relevant information (*i.e.* name of medication, total daily dose, unit, start and stop dates, reason for use if consistent with the definition of an AE) must be recorded. All changes in medication should be noted in the CRF.

10.2.8 *Baseline symptoms*

A baseline symptom is defined as an event that occurs between subject's signing of the ICF until the first administration of IP (i.e. an event that occurs during the screening period). Such events are not AEs and will be recorded in the medical history log in the eCRF.

10.2.9 *HIV and Hepatitis B/C*

Subjects will be tested for HIV and hepatitis B/C prior to inclusion into the study in order to protect personnel handling the blood samples.

10.2.10 *Pregnancy test*

All females of childbearing potential will do a pregnancy test (urine dipstick) at screening.

10.2.11 *Urine drug screen*

Urine will be screened for drugs of abuse at screening using the Alere™ Drug Screen Test Panel. Additional random tests can be performed during the study period.

10.2.12 *Alcohol breath test*

An alcohol breath test will be performed at screening. Additional random tests can be performed during the study period.

10.2.13 *Carbon monoxide measurement*

Measurement of carbon monoxide (CO) in exhaled air will be performed at visits to the clinic.

10.3 Assessments related to primary endpoints

10.3.1 *Pharmacokinetic samples and analysis*

Venous blood samples (approximately 5 mL) for the determination of plasma concentrations of nicotine after administration of the IP, will be collected through an indwelling venous catheter at the pre-specified time-points (see Table 8.1-1 in Section 8.1). The following time windows will apply for the PK sampling:

± 1 minute for time-points up to 30 minutes post-dose.

± 2 minutes for time-points up to 90 minutes post-dose.

± 5 minutes for time-points up to 6 hours post-dose.

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

The blood samples will be collected in pre-labelled tubes. All the collected blood samples will be centrifuged to separate plasma. The separated plasma from each blood sample will be divided into 2 aliquots in pre-labelled cryotubes and frozen at -20°C.

For further details see lab manual.

Plasma samples for determination of plasma concentrations of nicotine will be analysed by ABS Laboratories Ltd by means of a validated using LC-MS/MS method. Samples from all evaluable subjects will be analysed.

10.4 Assessments related to secondary endpoints

10.4.1 *Samples for analysis of Methyl salicylate*

Plasma samples for determination of plasma concentrations of nicotine will also be analysed for plasma levels of salicylic acid which is a proxy for plasma levels of the flavour component Methyl salicylate. The levels of salicylic acid will be used for read-across to assess the plasma levels of Methyl salicylate.

The analysis of salicylic acid will only be performed for samples drawn during the experimental session when the IP is containing the flavour component i.e. ZYN® Wintergreen 6 mg and Longhorn Wintergreen pouch.

Plasma samples for determination of plasma concentrations of salicylic acid will be analysed by ABS Laboratories Ltd by means of a validated using LC-MS/MS method. Samples from all evaluable subjects excluding withdrawn or dropout subjects will be analysed.

10.4.2 *Collection of used pouches*

Used pouches will be collected after 60 minutes (+/- 1 minute) of use for the determination of residual nicotine in the Investigational Products.

All the collected pouches will be frozen immediately at -20°C. Pouches for extraction of nicotine will be analysed by Swedish Match. Pouches from all evaluable subjects will be analysed.

10.5 Adverse events

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

10.5.1 *Definition of adverse event*

An AE is any untoward medical occurrence in a clinical study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including clinically significant abnormal values from relevant tests, such as clinical safety laboratory tests, vital signs), symptom, or disease temporally associated with the use of an IP, regardless of whether it is considered related to the IP.

A treatment emergent AE (TEAE) is any AE not present prior to the initiation of IP administration or any event already present that worsens in either intensity or frequency following exposure to the IP.

Only TEAEs are collected in this study (*i.e.* events occurring between screening and the first IP administration are regarded as *baseline symptoms* and should not be recorded in the AE log in the eCRF)

10.5.2 *Definition of serious adverse event*

An SAE is any AE that:

- results in death
- is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe)
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is medically important (this refers to an event that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent any of the SAEs defined above)

Examples of medically important events are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalisation, development of drug dependency, and drug abuse.

Planned hospitalisations or surgical interventions for a condition that existed before the subject/patient 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 viewpoint must be taken, and the AE must be reported as an SAE.

10.5.3 *Time period and frequency for collecting adverse events*

All AEs (including SAEs) will be collected from the start of intervention until the follow-up visit.

Any AE with start date on the day of first IMP administration must be recorded with start time.

At the Follow-up Visit, information on new AEs or SAEs, if any, and stop dates for AEs recorded and on-going during the dosing period must be recorded.

Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded in the Medical History Log of the eCRF, not in the AE Log.

Investigators are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

10.5.4 *Assessment of severity/intensity*

The grading of the severity/intensity of AEs will follow the CTCAE v4.03 [4]. 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 *severity/intensity* of an AE using the following definitions, and record it on the *Adverse Event Form* in the CRF:

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

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

10.5.4.1 Assessment of causal relationship

The Investigator must assess the causal relationship between an AE and the IP using the definitions below and record it the Adverse Event Log of the eCRF:

- *Probable* – the AE has a strong temporal relationship to the IP or recurs on re-challenge, and another aetiology is unlikely or significantly less likely
- *Possible* – the AE has a suggestive temporal relationship to the IP, and an alternative aetiology is equally or less likely
- *Unlikely* – the AE has no temporal relationship to the IP or is due to underlying/concurrent illness or effect of another drug (that is, there is no

causal relationship between the IMP and the AE).

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

10.5.4.2 Assessment of outcome

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

- *Recovered* – the subject has recovered completely, and no symptoms remain.
- *Recovering* – the subject's condition is improving, but symptoms still remain.
- *Recovered with sequelae* – the subject has recovered, but some symptoms remain (for example, the subject had a stroke and is functioning normally, but has some motor impairment).
- *Not recovered* – the subject's condition has not improved and the symptoms are unchanged (for example, an atrial fibrillation has become chronic).
- *Death*

10.5.5 ***Collecting 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

10.5.6 ***Recording adverse events***

AEs must be recorded in the AE Log of the eCRF. The investigator must provide information on the AE, preferably with a diagnosis or at least with signs and symptoms; start and stop dates, start and stop time; intensity; causal relationship to IP; 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.

If the severity/intensity of an AE increases a new AE Form must be completed in the eCRF.

10.5.7 ***Reporting of serious adverse events***

SAE reporting should be performed by the investigator within 24 hours of awareness via the eCRF or by e-mailing a copy of a paper SAE form. All available information regarding the SAE should be entered in the AE Log for the specific subject. By saving the event as "serious" in the eCRF, an e-mail alert is sent to predefined recipients to highlight that an SAE has been registered. [redacted]

The SAE report is reviewed by a designated person at CTC's Pharmacovigilance (CTC PV) to ensure that the report is valid and correct. For fatal or life-threatening SAEs where important

or relevant information is missing, immediate follow-up is undertaken and queries to the site are raised. Investigators or other site personnel should inform CTC PV of any follow-up information on a previously reported SAE immediately but no later than the end of the next business day of when he or she becomes aware of it.

If the SAE report in eCRF is updated, a new e-mail alert is sent to the predefined recipients.

The appointed Medical Monitor will provide his/her causality assessment and make an expectedness assessment once the report is judged to be complete.

If any additional documentation is required (e.g. autopsy report), CTC PV will request this information from the study site.

In case the eCRF cannot be accessed, the SAE should be reported by manually completing the paper SAE Form, provided in the Investigator Site File. The completed, signed and dated paper SAE Form should, within 24 hours, be scanned and e-mailed to:

[redacted]

A copy of the paper SAE form must also be e-mailed to CTC [redacted]

The study site should 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 should be reported electronically as well.

The Sponsor or delegate will assume responsibility for reporting SAEs to IECs in accordance with local regulations.

10.5.8 *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 final Follow-up visit, whichever comes first. At the Follow-up visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded. AEs assessed as stable by the Investigator at the last Follow-up 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.

SAEs spontaneously reported by a subject to the Investigator within 30 days after the last follow-up assessment must be handled in the same manner as SAEs occurring during the study. These SAEs will be reported to the Sponsor.

10.5.9 *Procedures in case of pregnancy*

In case of pregnancy or suspicion of possible pregnancy, the study treatment must be stopped immediately, and the subject discontinued from participation in the study. Pregnancy itself is not 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 and documented even after the subject was discontinued from the study.

All events of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as AEs. All outcomes of pregnancy must be reported to the Sponsor and the Principal Investigator on the pregnancy outcomes report form.

10.6 Treatment of overdose

An overdose is a dose in excess of the dose specified for each cohort in this CSP.

Over-dosing is not likely to occur in this study since all IP will be administered by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures should be adopted as required.

An overdose should be documented as follows:

- An overdose with associated AE is recorded as the AE diagnosis/symptoms in the AE Log of the eCRF.
- An overdose without associated symptoms is only reported in the subject´s medical records.

10.7 Appropriateness of measurements

The stopping rules for dose escalation used follows the recommendations and grading system of CTCAE v4.03 [4] but also take into account the recommendations published by M Sibille et al 2010 [5] which is an adaptation to FIH studies of the grading systems previously proposed by NCI [4], WHO [6], NIH [7] and FDA [8].

All other methods used for safety assessments are commonly used in standard medical care and in phase I clinical studies.

11 PROCEDURES FOR BIOLOGICAL SAMPLES

11.1 Sample collection for pharmacokinetic measurements

Blood samples will be drawn by venepuncture or an indwelling venous catheter into one 5 mL vacutainer tube at time points specified in the detailed Schedule of events in Section 8.1 (Table 3).

After PK blood collection, the vacutainer tubes will be inverted and centrifuged according to local standard procedures. The separated plasma is transferred into 1.8 mL cryo-tubes (in duplicate) and immediately placed at -20°C.

11.2 Volume of blood

The anticipated volume of blood samples collected during the study from each subject will not exceed 450 mL (*i.e.*, less than the volume drawn during a regular blood donation).

Approximate total volume per subject per type of samples:

Safety samples (microbiology)	5 mL
PK samples	350 mL

11.3 Handling, storage and destruction of laboratory samples

All biological samples will be registered in a tissue-bank at CTC (893).

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

The samples for analyses of PK parameters will be stored at -20°C until analysed. The samples will be disposed of after the CSR has been finalised.

All plasma samples transferred to the Sponsor's biobank will, if not used, be disposed of after 10 years.

11.4 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 subjects while in storage at the research clinic until shipment and keeps documentation of receipt of arrival.

The sample receiver (the analytical laboratory) keeps full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

11.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent, biological samples will be disposed of /destroyed, if not already analysed and documented.

The Principal Investigator will ensure that:

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

The Sponsor has to ensure that the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the research clinic and the action is documented.

12 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

12.1 QUALITY MANAGEMENT

12.1.1 *Critical process, system and data identification*

As this is the third study that Swedish Match AB performs in cooperation with CTC the critical processes, systems and data have been identified and the risks mitigated.

12.2 Quality assurance and quality control

The sponsor is responsible for securing agreements with involved subcontractors and to perform regular subcontractor oversight to ensure CSP compliance, GCP compliance and compliance with applicable regulatory requirements.

Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

13 ETHICAL AND REGULATORY REQUIREMENTS

13.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP) E6 (R2), EU Clinical Trials Directive, and applicable local regulatory requirements.

A link to the Declaration of Helsinki is included in Appendix 17.2.

13.2 Ethics and regulatory review

The Sponsor has delegated to CTC the responsibility for submission of study documents to the applicable CA according to local regulatory requirements.

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

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

13.3 Subject information and consent

It is the responsibility of the Investigator or an authorised associate to give each potential study subject (or the subject's legally acceptable representative and/or witness, as applicable) 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 emphasised that participation in the study is voluntary and that the subject/patient 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 the ICF.

Before performing any study-related procedures the ICF must be signed and personally dated by the subject (or their legally acceptable representative and/or witness, as applicable) and by the Investigator. A copy of the subject information including the signed 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 CRF. The subject information sheet and the signed 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.

13.4 Subject data protection

The ICF includes information that data will be recorded, collected and processed and may be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC) and General Data protection Regulation (GDPR), the data will not identify any persons taking part in the study.

The potential study subject (or the subject's legally acceptable representative and/or witness, as applicable) should be informed that by signing the ICF he/she approves that authorized representatives from Sponsor and CTC, the concerned IEC and CA have direct access to his/her medical records for verification of clinical study procedures. This agreement is to be substantiated in a separate document, according to local requirements.

The subject/patient has the right to request access to his/her personal data and the right to request rectification of any data that is not correct and/or complete in accordance with the European Union Data Protection Directive (95/46/EC) and the request will be raised to the Principal Investigator.

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

Personal data that are collected in the study such as health information and ethnicity are considered as sensitive personal data. This data will be pseudoanonymized, i.e. personally identifiable information (PII) 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 end, only anonymized data, i.e. aggregated data sets, can be used.

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

13.5 Changes to the approved clinical study protocol

Any proposed change to the approved Final CSP (including appendices) will be documented in a written and numbered clinical protocol amendment. All substantial amendments to the protocol must be approved by the appropriate IEC and/or CA before implementation according to applicable regulations.

13.6 Audits and inspections

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

13.7 Insurance

Subjects will be covered under Swedish match 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 services performed by CTC.

14 STUDY MANAGEMENT

14.1 Training of study site personnel

Before enrolment of the first study subject a Sponsor representative or delegate will perform a study initiation visit at the research clinic. 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) utilised.

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.

14.2 Clinical monitoring

The Sponsor is responsible for securing agreement from all involved parties to ensure direct access to all study related 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 plan, approved by the sponsor and provided separately, the responsible Monitor will periodically visit the study site at times agreed upon by the Investigator and the Monitor. At the time of each monitoring visit, the role of the Monitor is (but not limited to) to:

- 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 eCRF 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 Monitoring 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 study team members at CTC in accordance with the risk-based monitoring plan.

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

14.3 Source data documents

A separate *Source Data Verification List* will be generated for each site before start of enrolment, specifying the location of the source of derived information appearing in the eCRF. This document must be signed by the Principal Investigator and the Monitor to confirm agreement before start of recruitment.

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

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

14.4 Study agreements

The Principal Investigator must comply with all the terms, conditions, and obligations of the Clinical Study Agreement for this study.

Agreements between Sponsor and CTC must be in place before any study-related procedures can take place, or subjects/patients be enrolled.

14.5 Study time table and end of study

The study is expected to start in Quarter 4, 2018 and to be completed by Quarter 1, 2019.

A subject is considered to have completed the study if he/she has completed all visits in the study including visit 9, Follow up.

The end of the study is defined as the date of visit 9, Follow up.

The end of the clinical part of the study is defined as the last visit of the last subject participating in the study.

14.6 Discontinuation of the study

The Sponsor reserves the right to discontinue the study at any time, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must inform all participating subjects and perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused IP and other study materials must be returned and all CRFs completed as far as possible.

14.7 Reporting and publication

14.7.1 *Clinical study report*

A clinical study report (CSR), in compliance with ICH E3; *Structure and content of clinical study reports*, describing the conduct of the study, the statistical analysis performed and the results obtained, will be prepared by CTC. The report will be reviewed and approved by, as a minimum, the Principal Investigator, the Statistician and the Sponsor.

14.7.2 *Confidentiality and ownership of study data*

Any confidential information relating to the IP 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 belonging to the Sponsor.

14.7.3 *Publication*

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

14.8 Archiving

The Principal Investigator is responsible for maintaining essential documents, (as defined in ICH E6 GCP, Section 8) for 10 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 anonymous CRF data), the original signed ICFs and detailed records of disposition of IP.

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 Trial Master File in accordance with ICH E6 GCP, Section 8 and applicable regulatory requirements.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the clinic and filed in the Investigator Study File for archiving for 10 years after finalization of the CSR.

The completed original eCRFs are the sole property of the Sponsor and should 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.

15 DATA MANAGEMENT

The data management routines include procedures for handling of the eCRF, database set-up and management, data entry and verification, data validation, quality control (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 computerised online edit checks identifying e.g. data values that are outside the allowed range and SAS-programmed offline checks on data exports. All study-specific and standard data validation programming will be tested in a separate testing environment prior to use on production data.

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

15.1 The web based eCRF

All Clinical data will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by PCG Solutions 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 site before inclusion of the first subject (Section 14.3).

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

15.2 The entering of data into the eCRF

All entries, corrections, and alterations are to be made by the Investigator or assigned site staff. Neither the Monitor nor any other study team member besides site staff can enter data in the eCRFs. 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 should record such information in the eCRF. The Investigator will be required to electronically sign off the clinical data. This will be performed by means of the

Investigator's unique UserID and password; date and time stamps will be added automatically at time of electronic signature

15.3 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 risk-based monitoring plan.

If corrections are needed, the responsible Monitor or Data Manager will raise a query in the eCRF. An appropriate member of the site staff will answer the queries in the eCRF. This will be audit trailed electronically within the eCRF, meaning that the name of investigational personnel, time, and date is logged.

15.4 Audit trail

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

15.5 External data

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

15.6 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 start of coding). Prior and concomitant medications will be coded according to the World Health Organisation (WHO) Anatomic Therapeutic Chemical (ATC) classification system. All coding will be approved by Sponsor prior to database lock.

15.7 Database lock

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

16 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).

16.1 General

Pharmacokinetic data will be presented using summary statistics. Data will be presented in terms of number (N), arithmetic mean, standard deviation (SD), median, minimum and maximum value. In addition, for the parameters AUC and C_{max} 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 treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time.

The primary endpoint will be investigating a one sided hypothesis that ZYN 6mg has a lower AUC compared to 2 pouches of 8 mg Swedish snus, using a significance level of 2.5%. All other test will be two-sided with a significance level of 5%.

16.2 Determination of sample size

Former studies had showed that the AUC for the ZYN smooth 6mg has a AUC of 3581 and 2 pouches of 8 mg Swedish snus has a AUC of 5586 with a common standard deviation of 1920.

The hypothesis is that ZYN smooth 6 mg has a significant lower AUC compared with 2 pouches 8mg Swedish snus. With a power of 80% and a significance level of 2.5% will the number of subjects needed be 32, assuming 10% drop out rate a total of 36 subjects will be included.

16.3 Analysis data sets

16.3.1 *Full analysis set*

The Full Analysis Set (FAS) will consist of all subjects who have been randomised and received at least one dose of IP. This population will be used as the Safety analysis set.

16.3.2 *Per protocol set*

The Per Protocol Analysis Set (PPS) will consist of all subjects who have been randomised and completed the study without any major protocol deviations that are judged to compromise the analysis of the data. All protocol violations will be judged as major or minor at the clean file meeting. This population will be used as the PK analysis set.

16.4 Description of study population

16.4.1 *Demographics and baseline characteristics*

Descriptive statistics for demographics, weight and height will be presented by treatment.

16.4.2 *Medical/surgical history and prior/concomitant medication*

Medical/surgical history and prior/concomitant medications will be presented by treatment using descriptive statistics and listings.

16.4.3 *Treatment compliance*

The number of subjects treated in each treatment period and their individual dose will be tabulated.

16.4.1 *Physical examination*

Abnormal findings will be specified and presented by subject and summarised by treatment and period.

16.4.2 *Safety laboratory analyses*

Safety laboratory data will be presented by individual time courses for each parameter and subject and summarised by treatment and period.

16.5 Analysis of primary endpoints

16.5.1 *Comparison of total nicotine exposure (AUC_{inf}) after administration of ZYN® 6mg and 2 pouches of 8 mg Swedish snus*

AUC_{inf} based on plasma concentrations of nicotine after administration of one single dose of ZYN® Smooth 6 mg of nicotine, to that of one single dose 2 pouches of 8mg Swedish snus will be described using summary statistics and non-parametric signed Wilcoxon rank sum test for between treatment difference.

16.6 Analysis of secondary endpoints

16.6.1 *Comparison of PK profiles between all included products*

The PK analysis will be based on the PPAS and performed by CTC. The PK parameters will be calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin® version 6.3 or later (Pharsight Corporation, U.S.A.).

The following non-compartmental PK parameters will be assessed:

- T_{max} (sampling time at which C_{max} occurred)
- C_{max} (maximum observed concentration)

- AUC_{inf} (area under the curve from 0 to infinity)
- AUC_{last} (area under the curve from 0 to t hours where t is the last measured concentration)

The mean + SD of AUCinf based on plasma concentrations of nicotine after administration of each pouch, will be calculated. AUC_{0-t}, AUC_{inf}, Cmax, Tmax, and terminal half-life will also be calculated.

AUC_{0-t}, AUC_{inf}, Cmax, Tmax, and terminal half-life of all included products will be described using summary statistics and analyzed using signed Wilcoxon rank sum test for between treatment differences for all pairwise comparisons.

16.6.2 *In-vivo extracted amount of nicotine*

The mean + SD extracted dose of nicotine from each pouch, will be calculated. The extracted dose of nicotine will be analyzed using the signed Wilcoxon rank sum test for within subject difference.

16.6.3 *Correlation between extracted nicotine and AUCinf*

The correlation between the AUC and the total amount of nicotine extracted from the pouch will be analyzed using Proc corr. in SAS.

16.6.4 *Analysis of plasma levels of Methyl salicylate*

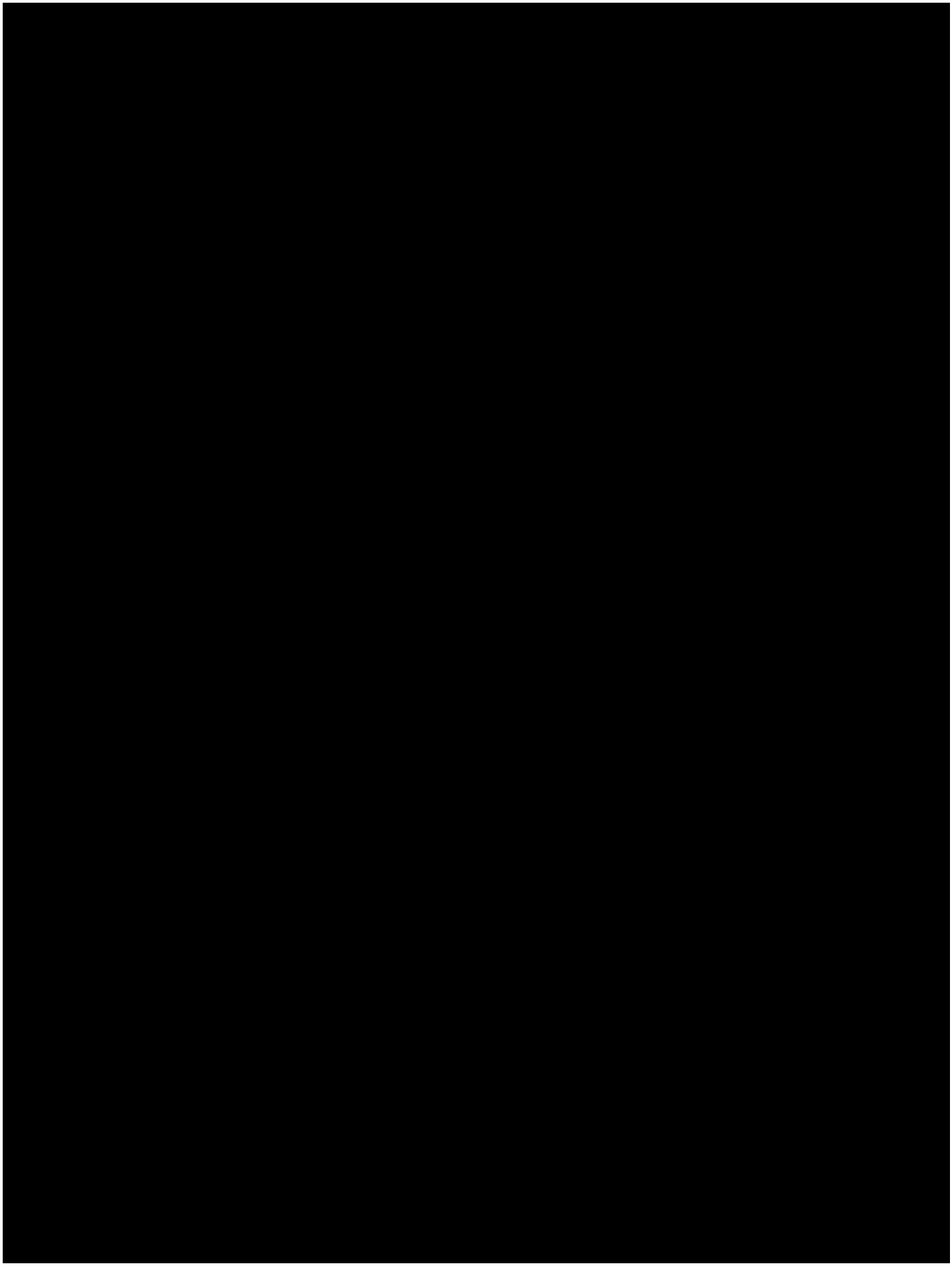
Plasma levels of Methyl salicylate will be summarized using descriptive statistics.

16.6.5 *Adverse events*

AE data will be summarised by System Organ Class (SOC) and PT. All AE data will be fully listed and include the verbatim term entered by the Investigator.

17 APPENDICES

17.1 Signatures



17.2 Declaration of Helsinki

http://www.up.ac.za/media/shared/Legacy/sitefiles/file/45/2875/declarationofhelsinki_fortaleza_brazil2013.pdf

18 REFERENCES

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3. Henningfield JE, Radzius A, Cooper TM, Clayton RR. Drinking coffee and carbonated beverages blocks absorption of nicotine from nicotine polacrilex gum. JAMA, 264 (1)560-4 (1990)
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