Date: 2013-06-17
Project: Swedish snus & sublingual nicotine

PRODUCTS: Swedish Portion Snus; PSWM 0.5 g (16 mg nicotine/g), PSWL 1 g (8 mg nicotine /g), PSWL 1 g (16 mg nicotine /g), PSWL 2x1 g (8 mg nicotine /g).
Nicorette® Microtab sublingual nicotine tablets, 6 mg.
PROTOCOL No: SM WS 12 **STATUS**: Final Version

EudraCT number 2011-006110-14

Study Report

Swedish Snus compared with an elevated dose of sublingual nicotine (Nicorette® Microtab).

Nicotine pharmacokinetics and subjective effects of single doses.

Date: 2013-06-17

2 ABSTRACT

Introduction: Previous studies of Swedish Portion Snus (SS) have indicated that the amount of extracted nicotine is generally not linear with pouch size, and it is unclear whether the amount of nicotine taken up into the blood circulation is linear with amount of extracted nicotine. Surface area, saliva penetration and diffusion factors may be as important determinants of nicotine uptake as nicotine content. The objective of this study was to determine nicotine absorption for some SS products in comparison with an over-the-counter sublingual nicotine tablet (Nicorette® Microtab) at an elevated dose.

Methods: We conducted an open-label, 5-way, crossover study involving 18 healthy snus users. One of 4 SS products was administered at each of 4 visits: 1 g SS (8 mg nicotine), 0.5g SS (8 mg nicotine), (1 g SS (16 mg nicotine), and two portions of 1 g SS (2 x 8 mg nicotine. Humidity and pH were kept constant. The study was partially randomized. This implies that the order of Snus administrations A,B,C,D was fully randomized, while the reference Nicotab® was tested separately at a fifth visit for practical (logistic) reasons. Blood samples were taken at intervals over 360 min and sensory perception was assessed by Visual Analogue Scale (VAS). Dose proportionality of AUC_{inf} (linear pharmacokinetics), as a measure of systemic exposure to nicotine, versus *In-vivo* extracted dose (linear pharmacokinetics) was investigated, based on pooled individual data on the four different snus products (B, C, D, E) for all 18 participants.

Results: For the 4 SS products, extracted amount of nicotine were ranked as follows: 1 g SS 8 mg; 1.56 ±0.95 mg, < 0.5g SS 8 mg; 1.90±0.82 mg, < 1 g SS 16 mg; 3.0 ±1.65 mg, < two portions of 1 g SS 2 x 8 mg; 3.0 ±1.35 mg.

Blood plasma levels of nicotine were ranked according to maximum plasma concentration (Cmax) of nicotine as follows: 1 g SS 8 mg; 12.2 ± 4.2 ng /mL, < 0.5g SS 8 mg; 9.0 ± 3.7 ng /mL, < 1 g SS 16 mg; 14.4 ± 5.5 ng/mL, < two portions of 1 g SS 2 x 8 mg; 17.7± 6.8 ng/mL.
The area under the plasma concentration–time curve (AUC_{inf}) were ranked as follows: 1 g SS 8 mg; 1.56 ±0.95 mg, < 0.5g SS 8 mg; 1.90±0.82 mg, < two portions of 1 g SS 2 x 8 mg; 2639.7± 879.7ng∗min/mL, < 1 g SS 16 mg; 2792.3± 1015.3 ng∗min/mL.

The inter-individual extraction showed quite high variability, from 10 to 57%. However, the intra-individual variability between the four different snus products (B, C, D, E) was rather small. The relationship between the AUC_{inf} and the *In-vivo* extracted dose per kg body weight showed fairly good linearity. The best fit was obtained in a logarithmic X-Y diagram, $p < 0.0001$. A good linearity was also obtained when AUC_{inf} was plotted against extracted dose/Body Surface Area (BSA) ($R^2=0.6632$).

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Nicotine was absorbed more slowly from 6 mg of Nicorette® Microtab sublingual nicotine tablets, but systemic exposure was within the range of the SS products (Cmax = 10.4±4.7 ng/mL ng.h/ml; AUC = 2976.3± 1491.8ng∗min/mL).

All products including Microtab increased "head rush" and reduced craving over the first 30 minutes when recordings were made. The effects were strongest for SS E (two 1 g SS portions, 2 x 8mg), however did not reach statistically significant difference versus Microtab, due to small sample size.

Conclusions: This study assessed nicotine extraction and systemic uptake from pouched SS products. The similar nicotine absorption for 1 g SS portions of 16mg and two 1 g SS portions, 2 x 8mg, indicates that absorption kinetics were dependent on total nicotine extraction rather than mode of administration.

The inter-individual extraction showed quite high variability, from 10 to 57%. However, the intra-individual variability between the four different snus products (B, C, D, E) was rather small. Each subject showed a high consistency with respect to extraction. Individual factors such as salivation and discreet oral movements may separate individuals, however were not possible to measure with the design used.

The relationship between the AUC_{inf} and the *In-vivo* extracted dose per kg body weight showed fairly good linearity. The extracted amount of nicotine from different preparations of pouched SS thus provides a good prediction of the systemic exposure to nicotine, when humidity and pH are identical. *In-vivo* extraction studies may therefore be valuable in product development.

The SS portions of a total of 16mg showed essential similarity, pharmacokinetic (AUC) as well as pharmacodynamic (VAS scores), to the 6 mg of Nicorette® Microtab sublingual tablets. According to the results of the present study, however, the Swedish Portion Snus products, 8mg as well as 16mg strengths, produced higher maximum blood nicotine concentrations in shorter time, compared to the Microtab.

Date: 2013-06-17

3 SUMMARY

Study code: SM WS 12, EudraCT number 2011-006110-14.

Title: Swedish Snus compared with an elevated dose of sublingual nicotine (Nicorette® Microtab). Nicotine pharmacokinetics and subjective effects of single doses.

Background & rationale: Swedish Snus (SS) is commonly used for smoking cessation in Sweden. Nicotine Replacement Therapy (NRT) when used as prescribed typically delivers less nicotine and at a slower rate than Swedish Snus. Also, surveys have shown that 10-15% of habitual users of pouched Snus products often use two pouches simultaneously, and the pinch size among users of loose Snus is typically larger than the standard 1.0 g pouch, on average 2.6 g (Digard H et al. 2009). Currently available NRTs when used as prescribed may simply deliver too little nicotine, which may explain the wide-spread use of Swedish Snus (SS) for smoking cessation. Results from studies of NRT among users of smokeless tobacco have also generally been modest (Hatsukami DK et al. 2000).

When comparing the nicotine content of different nicotine-containing products such as Swedish Snus (SS) and NRT, it is important to consider that the nicotine extraction and uptake vary considerably depending on product type and formulation. Nicotine is extracted and absorbed, both through the oral mucosa, and, after being mixed with saliva and swallowed, through the mucosa of the gastrointestinal tract. There it undergoes an extensive first-pass metabolism to mainly cotinine, in the liver.

With snus, previous studies have indicated that on average about 22-44% of the total nicotine content is extracted and 10 -20 % is absorbed, with large inter-individual variation (Lunell & Lunell 2005). Amount of extracted nicotine is generally not linear with pouch size (amount of tobacco): it is larger with small compared to large pouches, which suggests that surface area, saliva penetration and diffusion factors may be as important determinants of nicotine uptake as nicotine content. Commercially available snus products have a nicotine content ranging between 1 - 2%. It is not known if the nicotine uptake from snus is linear with nicotine content.

In view of these circumstances, we found it highly justified to study the nicotine delivery profile of some snus products in comparison with a NRT. We have previously conducted studies on snus products with

different nicotine content versus 2 and 4 mg nicotine chewing gum (Lunell E & Lunell M 2005, Lunell E & Curvall M 2011). We now extend those observations by comparing Swedish snus (SS) with nicotine sublingual dissolvable tablets at an elevated dose, 6mg.

Primary Objective:

To compare each subject's plasma concentration of nicotine, C_{max}, T_{max} and AUC_{inf} of one single dose of 1 g Swedish Snus (16 mg nicotine) to that of a single dose of 6 mg of sublingual nicotine tablets (Nicorette® Microtab).

Secondary Objectives:

To compare plasma concentrations of nicotine, Cmax, Tmax, AUCinf of one single dose of 0.5 g portion SS containing 8 mg nicotine with a single dose of 1 g portion SS containing 8 mg nicotine and to compare a single dose of 1 g portion SS containing 16 mg nicotine with a single dose of 2 x 1 g portion SS containing 2 x 8 mg nicotine.

To compare the *in-vivo* extracted dose of nicotine from each portion of snus, with that of the Nicorette sublingual tablet. An assumption was made that the total 6 mg amount of nicotine from the Nicorette® Microtab) was extracted, since the tablets are completely dissolved in the mouth.

To assess dose proportionality (linear pharmacokinetics) of nicotine after administration of one single dose of four different snus products.

To compare each subject's rating of the subjective effects -craving intensity, overall "product strength" (head rush), increased salivation, burning sensation in the mouth and/or throat, using a Visual Analogue Scale (VAS) anchored with "not at all" to "extremely" at the time points of blood sampling up to 30 minutes. A recent analysis showed that a ten-item questionnaire (QSU-brief) is not more sensitive to abstinence or reliable than a single rating of craving (West R & Ussher M 2010).

Total sample size:

The study included 16 subjects who completed all treatments. Linear pharmacokinetics has been shown for buccal administration of nicotine in the dose interval 1-4 mg [Molander L and Lunell E 2001]. A previous study [Lunell E & Curvall M 2011] made a calculation of sample size possible. Nicotine extraction from 1 g SS containing 8 mg nicotine/pouch was estimated at 2.18±0.92 mg per 1 g portion. Under the assumption of a complete disintegration and extraction of the 6 mg of Nicorette sublingual nicotine tablets versus the 2.18±0.92 mg nicotine, extraction levelling off

above the 8 mg strength for the SS and a standard deviation of 5.0 the estimated sample size is 16 with a power of 80% and alpha=0.05.

Study design: Open, partially randomized, cross-over. Products tested were 0.5g portion SS (8 mg nicotine), 1 g portion SS (8 mg nicotine) and 1 g portion SS (16 mg nicotine), as were two pouches of 1 g portion SS (2 x 8 mg nicotine). The four SS B, C, D and E were tested in random order, while the 6 mg (3 x 2mg nicotine) of Nicorette® Microtab was tested separately for practical (logistic) reasons. Single dose administration. Subjects reported to the laboratory for five experimental sessions. After baseline measurements, plasma nicotine concentrations were monitored over 6 hours.

Subject population: Sixteen male and 2 female (non-pregnant), healthy, non-smoking volunteers, 18-50 years of age, using 12.8±10.0 pouches of SS per day. Smokers were defined as "smoking during the last 24 hours according to self report and CO in exhaled air >10 ppm at the health check". Subjects were fasting overnight and abstinent from snus and all other nicotine containing products from 8.00 p.m. the night before each study session.

Test articles:
B: Swedish Portion Snus PSWM 0.5 g (16 mg nicotine/g)
C: Swedish Portion Snus PSWL 1 g (8 mg nicotine /g)
D: Swedish Portion Snus PSWL 1 g (16 mg nicotine /g)
E: Swedish Portion Snus PSWL 2x1 g (8 mg nicotine /g)

Note: pH and humidity of the various types of SS were kept constant.

Reference article: A: 6 mg dose of sublingual tablets (Nicorette® Microtab) (=3 tablets).

Batch No.: PH 127 F. Expiration Date 08/2015.

Procedure: The treatments were given as single doses in partially randomized order, except the reference 6 mg of Nicorette® Microtab was tested on a separate occasion for practical (logistic) reasons. The subject kept the three sublingual tablets still under the tongue for 30 minutes.

The subjects kept the portion(s) of snus still between the upper lip and the gum for 30 minutes. When two portions at one time were administrated they were placed one portion on each side of the mouth.

The subjects were not allowed to eat or drink coffee and carbonated beverages or any other liquid for the first 60 minutes after dose administration [6].

Serial blood samples were drawn before, and at regular time intervals up to 6 hours after start of administration (13 samples). Before entry to the study subjects underwent screening evaluations including medical history, physical examination, laboratory tests and electrocardiogram.

Study parameters:

Amount of nicotine extracted, plasma nicotine concentrations, C_{max}, T_{max} and AUC_{inf} for each treatment. The AUC_{inf} was based on plasma data corrected for background nicotine (time zero sample).

Each subject's rating of subjective effects using a Visual Analogue Scale (VAS), anchored with "not at all" to "extremely". VAS scores were obtained at the plasma concentrations sampling time points up to 30 minutes for:

- craving intensity
- overall "product strength" (head rush, "buzz", "hit")
- increased salivation
- burning sensation in the mouth and/or throat

Analysis:

The sample size calculation was based on the comparison between 1 g SS (16 mg nicotine), single dose, and 6 mg of sublingual nicotine tablets. Primary objective was to compare AUC_{inf} after administration of a single dose of 6 mg Nicorette® Microtab to that of one single dose of SS containing 16 mg nicotine (Snus D). Secondary objectives were to compare AUC_{inf} of Snus E to AUC_{inf} of Snus D and to compare AUC_{inf} Snus B to AUC_{inf} of Snus C. A secondary objective was also to assess dose proportionality (linear pharmacokinetics of AUC_{inf}) of nicotine for the three different snus products (B, C, D, E). The correlation between extracted amount of nicotine and the pharmacokinetic parameter AUC_{inf} was investigated. Assessment of dose linearity was based on pooled data on the different snus products.

The quotients of AUC_{inf} and C_{max} of the various snus products were analyzed with ANOVA with dose, period and subject as independent variables.
The confidence intervals were calculated with error taken from the ANOVA. Although not used for assessment of dose proportionality also C_{max} (secondary parameter) was tested.

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Results:

The mean (±SD) extracted amount of nicotine from Snus C, PSWL 1 g (8 mg nicotine/g), was estimated at 1.56 ±0.95 mg nicotine/portion. The mean (±SD) extracted amount of nicotine from Snus B, PSWM 0.5 g (16 mg nicotine/g), containing half the amount of tobacco compared to Snus C, was 22% larger, 1.90±0.82 mg nicotine/portion. The mean (±SD) extracted amount of nicotine from Snus D, PSWL 1 g (16 mg nicotine/g), 3.0 ±1.65 mg, was approximately the same as that from Snus E, PSWL 2x1.0 g (8 mg nicotine/g) 3.0 ±1.35 mg, although the 16 mg was divided into two 1g portions.

Mean (±SD) *in-vivo* disintegration time of 3 Nicorette® Microtab (=6mg) was 58.4 ±12.3 minutes (range 30-80 minutes). This was longer than expected and may have been due to formation of a larger aggregate in the oral cavity of the subjects. Subjects were instructed to keep the tablets still and not to suck on or chew them.

The extracted amount of nicotine was mirrored in the various AUC_{inf} values. The AUC_{inf} of the Snus D, PSWL 1g (16 mg nicotine /g) and Snus E, PSWL 2x1 g (8 mg nicotine /g) were 2792.3± 1015.3 ng∗min/mL and 2639.7± 879.7 ng∗min/mL, respectively. This corresponded to 93.8% and 88.7%, respectively, of the AUC_{inf} of 6 mg of Nicorette® Microtab, 2976.3± 1491.8ng∗min/mL.

The mean AUC_{inf} of Snus B, PSWM 0.5 g (16 mg nicotine/g), was 37 % larger, compared to that of Snus C, PSWL 1 g (8 mg nicotine /g). The mean AUC_{inf} of Snus B, PSWM 0.5 g (16 mg nicotine/g) and Snus C, PSWL 1 g (8 mg nicotine /g), were 2032.7± 724.5ng∗min/mL and 1484.4± 605.3 ng∗min/mL, respectively. This corresponded to 68.3% and 49.9%, respectively, of the AUC_{inf} of 6 mg of Nicorette® Microtab. The 8 mg dose of nicotine, thus seemed more bioavailable from the lower volume of tobacco.

A similar pattern was seen for C_{max}. The mean C_{max} of Snus B, PSWM 0.5 g (16 mg nicotine/g) was 34% larger than in Snus C although the products contained the same amount of nicotine. The C_{max} of Snus B, PSWM 0.5 g (16 mg nicotine/g), and Snus C, PSWL 1 g (8 mg nicotine /g), were 12.2 ± 4.2 ng /mL and 9.0 ± 3.7 ng /mL, respectively. This corresponded to 117% and 87%, respectively, of the C_{max} of 6 mg of Nicorette® Microtab, 10.4±4.7 ng/mL.

The mean C_{max} of Snus D, PSWL 1 g (16 mg nicotine /g) and Snus E, PSWL 2x1 g (8 mg nicotine /g) , were 17.7± 6.8 ng/mL and 14.4 ± 5.5 ng/mL, respectively. This corresponded to 170% and 138% of the mean C_{max} of 6 mg of Nicorette® Microtab.

For comparison of AUC_{inf} after administration of a single dose of 6 mg of sublingual nicotine tablets (Nicorette Microtab®) to that of one single dose of SS containing 16 mg nicotine (Snus D) a bioequivalence test was used. The geometric mean and 90% confidence interval of the AUC_{inf} ratio of the sublingual tablet and the Snus D were estimated at 0.962 (0.758 - 1.221). From these results it may be concluded that the two formulations were not bioequivalent according to strict criteria (90% C.I. of AUC_{inf} ratio within 0.80-1.25) with respect to AUC_{inf}, however very close to bioequivalence.

A bioequivalence test was also used to compare AUC_{inf} of Snus D (16 mg nicotine) to AUC_{inf} of Snus E (2x8mg nicotine). The geometric mean and 90% confidence interval of the AUC_{inf} ratio of the Snus D and Snus E were estimated at 1.049 (0.942 - 1.168). From these results it may be concluded that the two formulations were bioequivalent with respect to AUC_{inf}.

The same test was used also to compare AUC_{inf} of 0.5 g portion SS containing 8 mg nicotine (Snus B) to AUC_{inf} of 1 g portion SS containing 8 mg nicotine (Snus C). The geometric mean and 90% confidence interval of the AUC ratio of the Snus B and Snus C were estimated at 1.393 (1.273 - 1.524). Portion SS 0.5 g containing 8 mg nicotine (Snus B) thus displayed a higher bioavailability of nicotine compared to 1 g portion SS containing 8 mg nicotine (Snus C), measured as AUC_{inf}.

VAS scores of subjective effects (craving, head rush) for the examined Swedish Snus products showed great similarities to 6 mg of Nicorette® Microtab, however the statistical analysis showed significantly higher head rush values for Snus E compared to Snus B at time point 8 minutes and 16 minutes, p-values 0.0439 and 0.0225, respectively. For the comparison between Snus E and Snus C there were significantly higher values for Snus E at time point 30 minutes, p-value 0.0232. Statistical analyses are shown in Section 18.

There were no differences in adverse event rates between the three groups. VAS scores for increased salivation and burning sensation in the mouth and/or throat were higher for 6 mg of Nicorette® Microtab, compared to all Swedish Snus products, however not statistically significant.

Conclusion: One portion of 1g Swedish Snus (Snus D) containing (16 mg nicotine) and two portions of 1 g Swedish Snus (Snus E) (2x8 mg

nicotine) were essentially similar to 6 mg of Nicorette® Microtab both with respect to nicotine plasma concentration-time curves and to VAS scores for craving and head rush. Thus, it could be concluded that the SS containing 16 mg nicotine (Snus D and E) were very close to bioequivalent to 6 mg of Nicorette® Microtab with respect to the nicotine uptake measured as AUC_{inf}. However, C_{max} of Snus D and Snus E, respectively, were 170% and 138% of the C_{max} of 6 mg of Nicorette® Microtab, demonstrating a comparatively faster uptake.

The mean extracted amount of nicotine from Snus B, contained in half the amount of tobacco (0.5 g) was 22% larger, AUC_{inf} was 35% larger , and C_{max} was 34% larger, compared to 1 g portion Snus C, both containing 8 mg nicotine. It could thus be concluded that extraction as well as uptake and rate of uptake seemed to be better from the smaller pouches of snus.

The mean (±SD) extracted amount of nicotine from Snus D, containing 16 mg nicotine in one 1 g pouch, as well as uptake, were very similar to when the 16 mg was divided into two 1g portions (Snus E), placed on both sides under the upper lip.

The high dose of Swedish Snus containing 16 mg nicotine in one 1 g pouch was very well tolerated, as were all other Swedish Snus products. VAS scores for increased salivation and burning sensation in the mouth and/or throat were higher for 6 mg of Nicorette® Microtab sublingual nicotine tablets compared to all test preparations of Swedish Snus.

Finally, the present study showed fairly linear relationship between the AUC_{inf} and the extracted dose per kg body weight. It can therefore be concluded that *In-vivo* extracted dose offers a good guidance for prediction of nicotine uptake into the blood circulation in the development of new Snus products, compared to the nicotine content of each portion.

4 TABLE OF CONTENTS

5 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
BSA	Body Surface Area
CI	Confidence Interval
CRF	Case Reported Form
ICH	International Conference of Harmonization
Microtab	Nicorette® Microtab sublingual nicotine tablets
NRT	Nicotine Replacement Therapy
NA	Not applicable
NS	Nasal Spray
OPP	Oromucosal powder in pouch
OR	Odds Ratio
PRO	Patient-reported outcome
PSWL	Portion Snus White Large
PSWM	Portion Snus White Mini
SAE	Serious Adverse Event
SAER	Serious Adverse Event Reported
SDV	Source document verification
SS	Swedish Snus
VAS	Visual analogue scale

6 INVESTIGATORS AND STUDY ADMINISTRATION

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7 ETHICS

7.1 Ethical Conduct of the Trial

The trial was performed in accordance with the recommendations guiding physicians in biomedical research involving human Subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964 and later revisions, ICH guidelines and GCP.

7.2 Independent Ethics Committee (IEC)

The clinical study conduct lasted May 2012 – August 2012. Please see Section 21. In accordance with the Declaration of Helsinki, the study was approved by the Institutional Review Board of Lund University and written informed consent was obtained from all subjects prior to enrolment in the study.

7.3 Patient Information and Consent

It was the responsibility of the investigator to give each patient prior to inclusion in the trial, full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. The subjects were informed about their right to withdraw from the trial at any time. Written patient information (Please see Section 19) was given to each patient before enrollment. The written patient information was not changed without prior discussion with CROel AB. Furthermore, it was the responsibility of the investigator to obtain signed informed consent from all subjects prior to inclusion in the trial (Please see Section 19).

8 INTRODUCTION

8.1.1 Background

Sweden displays the lowest incidence of smoking among men in Europe. One explanation for the low incidence of smoking among men in Sweden may be that Swedish Snus, a form of smokeless tobacco or moist snuff with rapid oromucosal nicotine uptake, is used as a last resort for people who have failed stopping smoking with the available smoking cessation aids, such as Nicotine Replacement Therapy (NRT). Moist snuff products are capable of rapidly delivering nicotine to the bloodstream (Fant R V et al. 1999), and therefore may be more satisfactory than e.g. NRT products. In a survey using data from a representative sample (n = 6700) of the Swedish population aged 16–79 years, collected in 2001–2002, 62

percent of ex-smokers stated that they used snus as a cessation aid, compared with 38% who mentioned using nicotine replacement therapy (Ramstrom L 2003).

8.1.2 Study design

The study had an open, cross-over, partially randomized design. The sequence of treatments was partially randomized. The SS products B, C, D and E were thus given as single administrations randomly on four separate occasions, while 6 mg of Nicorette® Microtab was tested separately for practical (logistic) reasons. A 48 hour wash-out period was inserted between treatments. The Swedish Portion Snus PSWM 0.5 g (16 mg nicotine/g) = B, Swedish Portion Snus PSWL 1.0 g (8 mg nicotine /g) = C, Swedish Portion Snus PSWL 1.0 g (16 mg nicotine /g) = D, Swedish Portion Snus PSWL 2x1.0 g (8 mg nicotine /g) = E, were randomly tested. It should be noted that pH and humidity of the various types of SS were kept constant.

Serial blood samples were drawn for determination of nicotine levels before, during and after drug administration. The clinical study was designed, implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practise, with applicable local regulations (including European Directive 2001/20/EC) and with the ethical principles laid down in the Declaration of Helsinki.

8.1.3 Rationale and aim of the Study

Swedish Snus (SS) is commonly used for smoking cessation in Sweden. Nicotine Replacement Therapy (NRT) when used as prescribed typically delivers less nicotine and at a slower rate than Swedish Snus. Also, surveys have shown that 10-15% of habitual users of pouched Snus products often use two pouches simultaneously, and the pinch size among users of loose Snus is typically larger than the standard 1.0 g pouch, on average 2.6 g (Digard H et al. 2009). Currently available NRTs when used as prescribed may simply deliver too little nicotine, which may explain the wide-spread use of Swedish Snus (SS) for smoking cessation. Results from studies of NRT among users of smokeless tobacco have also generally been modest (Hatsukami DK et al. 2000).

When comparing the nicotine content of different nicotine-containing products such as Swedish Snus (SS) and NRT, it is important to consider that the nicotine extraction and uptake vary considerably depending on product type and formulation. Nicotine is extracted and absorbed, both through the oral mucosa, and, after being mixed with saliva and

swallowed, through the mucosa of the gastrointestinal tract. There it undergoes an extensive first-pass metabolism to mainly cotinine, in the liver.

With snus, previous studies have indicated that on average about 22-44% of the total nicotine content is extracted and 10 -20 % is absorbed, with large inter-individual variation (Lunell & Lunell 2005). Amount of extracted nicotine is generally not linear with pouch size: it is larger with small compared to large pouches, which suggests that surface area, saliva penetration and diffusion factors may be as important determinants of nicotine uptake as nicotine content. Commercially available snus products have a nicotine content ranging between 1 - 2%. It is not known if the nicotine uptake from snus is linear with nicotine content or with extracted amount of nicotine.

The hypothesis for the present study was that there should be a faster absorption of nicotine, to a higher peak blood concentration, from 1 g Swedish Portion Snus (8 and16 mg nicotine, respectively) compared to 6 mg of sublingual nicotine tablets. A comparison of the characteristics of the blood nicotine curves therefore seemed warranted.

Our aim was to compare extracted dose, dose proportionality (linear pharmacokinetics), subjective effects, C_{max}, T_{max} and AUC_{inf} of four different Swedish Snus products) to 6 mg of Nicorette® Microtab. The rationale for the choice of the 6 mg dose of the Microtab sublingual tablet is that it was the highest oral NRT dose that had proved safe (Molander L and Lunell E 2001). Secondly, NRT results among users of smokeless tobacco have generally been modest (Hatsukami DK et al. 2000).

9 STUDY OBJECTIVES AND ENDPOINTS

9.1 Primary Objective and Endpoint

The primary objective of the present study was to compare each subject's plasma concentration of nicotine, C_{max}, T_{max} and AUC_{inf} of one single dose of 1 g Swedish Snus (16 mg nicotine) to 6 mg of Nicorette® Microtab.

9.2 Secondary Objectives and Endpoints

To compare plasma concentrations of nicotine, C_{max}, T_{max}, AUC_{inf} of one single dose of 0.5 g portion SS containing 8 mg nicotine with a single dose of 1 g portion SS containing 8 mg

nicotine and to compare a single dose of 1 g portion SS containing 16 mg nicotine with a single dose of 2 x 1 g portion SS containing 2 x 8 mg nicotine.

To compare the *in-vivo* extracted dose of nicotine from each portion of snus, with that of the Nicorette sublingual tablet. An assumption was made that the total 6 mg amount of nicotine from the Nicorette® Microtab was extracted, since the tablets are completely dissolved in the mouth.

To assess dose proportionality (linear pharmacokinetics) of nicotine after administration of one single dose of four different snus products.

To compare each subject's rating of the subjective effects -craving intensity, overall "product strength" (head rush), increased salivation, burning sensation in the mouth and/or throat, using a Visual Analogue Scale (VAS) anchored with "not at all" to "extremely" at the time points of blood sampling up to 30 minutes. A recent analysis showed that a ten-item questionnaire (QSU-brief) is not more sensitive to abstinence or reliable than a single rating of craving (West R & Ussher M 2010).

10 INVESTIGATIONAL PLAN

10.1 Overall Study Design and Plan - Description

This study was a phase 1, single centre, open, partly randomised, controlled, single dose study, comparing five different treatments.

EVENT	Clinical examination ECG and lab. tests	INFORMED CONSENT	TABLET OR SWEDISH SNUS ADMIN.	PLASMA SAMPLING	VAS AND AE INTERVIEW
Screening	✓	✓			
Session 1			✓	✓	✓
Session 2			✓	✓	✓

Session 3			✓	✓	✓
Session 4			✓	✓	✓
Session 5			✓	✓	✓

A minimum period of 48 hours was kept between the five sessions.

10.2 Discussion of Study Design, Including the Choice of Control Groups

10.2.1 Study Design

The 4 mg Nicorette® Microtab sublingual nicotine tablets is currently the oral NRT with the highest content of nicotine approved on the market. The elevated dose of 6 mg of Nicorette® Microtab therefore appeared to be the most relevant comparison to 1 g Swedish Snus (16 mg nicotine). Additional comparisons with other doses (8 mg nicotine) and modes of administration were included. Treatments were allocated according to a randomization list generated by an independent organization (APL, Stockholm, Sweden) using "Design Algorithm" version 990418 in blocks of 9.

10.2.2 Screening visit

At this visit eligibility to participate in the study was checked. The subject was given information about the study procedures and signed the informed consent form.

At this visit the following procedures were conducted and documented:

- Informed consent
- Inclusion/Exclusion criteria
- Demographic data
- Body height and weight
- Supine blood pressure and heart rate
- Medical history

- Concurrent diseases/symptoms
- Concomitant medication
- ECG
- Physical examination
- Urinalysis
- Blood samples drawn for clinical chemistry (Na, K, Creatinine, ASAT, ALAT), hematology (Hb, WBC, Platelets) and virology (HIV, hepatitis).

If the subject fulfilled all criteria for enrolment, appointments for visits 1-3 were booked.

10.2.3 Consecutive visits after Randomization *(Visits 1-5)*

At every visit the following procedures were conducted and documented:

Predose

- Confirmation of eligibility - Exhaled Carbon Monoxide (ECO)

To confirm that each subject has abstained from smoking a test for exhaled carbon monoxide (ECO) is performed at each visit. Levels of ECO up to 13 ppm were considered compatible with abstinence.

- Concomitant medication
- An intravenous cannula was administered for the blood sampling

Blood sample (5 ml) for PK analysis (baseline=0 minutes).

Administration of investigational drugs

Investigational drugs were: 0.5 g portion SS containing 16 mg nicotine /g, 1 g portion SS (8 mg nicotine /g), 1 g portion SS (16 mg nicotine/g), 2 x 1 g portion SS (8 mg nicotine /g) and 6 mg of Nicorette sublingual nicotine tablets, respectively**.**

Blood sampling

Venous blood samples (5 ml) were collected in sodium heparinized Venoject glass tubes from an antecubital vein at the following time-points:

before (0), 2, 4, 8, 16, 24, 30, 45, 60 and 90 minutes and 2, 4 and 6 hours after drug administration.

Only non-smoking personnel were allowed to perform practical functions in this study. The blood samples were centrifuged within 30 minutes at 1000g for 10 minutes at ambient room temperature. The plasma was then separated and transferred to cryotubes, which were immediately frozen and kept frozen (-20ºC) pending analysis. The plasma samples were shipped by courier door to door on ice to ABS Laboratories, London, England.

10.2.4 Study subjects - Selection of Study Population

10.2.4.1 Inclusion Criteria

1. Consent to participate voluntarily and sign Informed Consent Form prior to any study procedure.
2. Healthy male/female, age 18 through 50 years. Female using contraceptive pill or negative pregnancy test.
3. Willing and able to comply with study procedures.
4. Snus user, minimum 12 pouches snus per day of pouched portion snus, minimum 1 gram/portion or half a can of loose snus.
5. Abstinent from any form of nicotine use from 8.00 p.m. the day before each trial day.
6. Fasting overnight from 11.00 p.m.

10.2.4.2 Exclusion Criteria

Subjects fitting any of the following characterizations were excluded from the study:

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. Second or third degree AV block or sick sinus syndrome; congestive heart failure classified as functional Class III or IV by the New York Heart Association; myocardial infarction within six months of baseline; a prolonged QTc interval at screen or pretreatment (defined as a QTc interval of > 450 msec for males or > 470 msec for females); other clinically significant heart conditions which would negatively impact on the subject completing the study.
3. Subjects with clinically significant liver disease which may prevent the subject from completing the study and/or an elevation in total bilirubin, alkaline phosphatase, LDH, ASAT, or ALAT of > 3 times the upper limit of the laboratory reference interval.
4. Subjects with clinically significant renal disease which may prevent the subject from completing the study and/or an elevation in serum creatinine of > 1.5 times the laboratory reference.

5. Surgery within 6 months of the Baseline visit that, in the opinion of the investigator, could negatively impact on the subject's participation in the clinical study.
6. Subjects who have participated in other drug studies within 30 days prior to enrolment.
7. Subjects with any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the drug.
8. Subjects who are using drugs capable of inducing hepatic enzyme metabolism within the previous 30 days (or 5 half lives of inducing agent, whichever is longer) of enrolment in this study.
9. Subjects with a medical history of seizures.

10.2.5 Removal of Subjects from Therapy or Assessment

A subject should be withdrawn from the study treatment if, in the opinion of the Investigator, it is medically necessary, or if it is the wish of the subject. In any circumstance, subject outcome should be documented and a Study Termination Report completed.

The subjects should abstain from nicotine for at least 12 hours prior to administration of each investigational drug and during the whole of the experimental session. Smokers were not admitted. Since Ex-smokers were admitted and could be tempted to cheat by smoking, the level of ECO was checked. A subject displaying an ECO level above 2-3 ppm (non-smoker level) was therefore sent home and asked to come back for a new session.

Previous experience has shown that subjects that have abstained from smoking for 12 hours have a plasma nicotine value of <4 ng/ml. Subjects with levels exceeding 4 ng/ml were therefore excluded from analysis.

A patient discontinued due to an adverse event was not replaced. If a patient discontinued the study for a reason other than an adverse event, the patient could be replaced.
The reason for withdrawal should be clearly described and the subject should, whenever possible, irrespective of the reason for withdrawal, as soon as possible be examined. Relevant samples should be obtained and all relevant assessments should be completed, preferably according to the schedule for the final assessment. The Case Report Form should be completed as far as possible and collected by the Monitor.

10.3 Treatments

10.3.1 Treatments Administered

Test articles:

B: Swedish Portion Snus PSWM 0.5 g (16 mg nicotine/g)

C: Swedish Portion Snus PSWL 1.0 g (8 mg nicotine /g)

D: Swedish Portion Snus PSWL 1.0 g (16 mg nicotine /g)

E: Swedish Portion Snus PSWL) 2x1.0 g (8 mg nicotine /g), placed on both sides under the upper lip.

Note: pH and humidity of the various types of SS were kept constant.

Reference article:

A: 6 mg dose of Nicorette® Microtab sublingual nicotine tablets (=3 tablets).

Batch No.: PH 127 F. Expiry date 08/2015.

10.3.2 Treatment Schedule

The treatments were given as single doses in randomized order. The subjects kept the sublingual tablets still under the tongue for 30 minutes.

The subjects kept the portion(s) of snus still between the upper lip and the gum for 30 minutes. For Snus E the two pouches were placed on both sides under the upper lip.

The subjects were not allowed to eat or drink for the first 60 minutes after each dose administration (Henningfield JE et al. 1990).

10.3.3 Method of Assigning Subjects to Treatment Groups

The subjects were assigned to Treatment Groups by a computer generated randomization list. The 6mg of Nicorette® Microtab were tested separately for practical (logistic) reasons.

10.3.4 Selection of doses in the study

The rationale for the choice of the 6 mg dose of the Nicotab sublingual tablet is that it was the highest oral NRT dose that had proved safe (Molander L and Lunell E 2001).

10.3.5 Timing of dose

A single dose was given approximately 8.00 a.m. on each study day.

10.3.6 Blinding

The present study was a single blind study with respect to single snus treatments. Subjects were administrated each dose by the personnel according to the randomization list.

10.3.7 Delivery of test drugs

The 1 g portion SS products (8 and 16 mg nicotine, respectively) and 0.5 g portion SS (8 mg nicotine) were delivered in identical food approved containers labeled with unique identification numbers from Swedish Match North Europe AB, Sweden. Nicorette® Microtab sublingual nicotine tablets, containing 2 mg nicotine/tablet were delivered in their original packs as available on the open market in Sweden from the pharmacy.

10.3.8 Labeling

The responsible pharmacist made individual packaging at CROel AB, Helsingborg, Sweden.

Labelling was in Swedish: 1 g Swedish Portion Snus (16 mg and 8 mg nicotine, respectively), 0.5 g Swedish Portion Snus (8 mg nicotine), and 6 mg Nicorette® Microtab.

For clinical trial.

CTN: SM WS 12
Subject No.: 1 (-18)
Treatment.: A (B,C, D, E)
Batch No.:
Expiry date:
Dosage: according to physician's instruction.
Responsible investigator: Erik Lunell, M.D. Carema AB, Eslöv
Keep out of reach of children.

10.3.9 Prior and Concomitant Therapy

Oral contraceptives were allowed. OTC drugs were allowed up till 24 hours before and after each dose of study medication. No prescription drugs or herbal remedies were allowed. Any such use in between study days should be reported to the investigator. Concomitant prescription drugs during the study considered necessary for the subject's welfare were allowed at the discretion of the investigator. All concomitant medication was recorded in the appropriate section of the CRF**.** No other drug under investigation was allowed concomitantly with the study drug. The subjects were not allowed to participate concurrently in any other study.

10.3.10 Treatment Compliance

Each dose was taken under supervision of the staff at the trial site. To confirm that each subject had abstained from smoking a test for exhaled carbon monoxide (ECO) was performed at each visit. Levels of ECO above 2-3 ppm (non-smoker level) were not considered compatible with abstinence [3?].

10.3.11 Emergency procedure

The Investigator was responsible for assuring that there were procedures and expertise available to cope with medical emergencies during the study.

10.3.12 Product accountability

All trial medication was accounted for and any discrepancies explained. The investigator was responsible for keeping detailed records. A product accountability form was available in the CRF to document the dispensed medication for each subject.

Trial medication was released from Swedish Match North Europe AB, Stockholm, Sweden, to the Investigator when all necessary approvals had been granted. Date, amount subject numbers and investigator or pharmacist’s signature were recorded on the trial product inventory. At the end of the trial, any unused trial medication was returned to Swedish Match AB according to a written agreement. Destruction and return of product and its documentation was the responsibility of CROel AB.

10.4 Efficacy and Safety Variables

10.4.1 Efficacy and Safety Measurements Assessed and Flow Chart

Subjective effects: Each subject's rating of product "strength" using a Visual Analogue Scale (VAS), anchored with "not at all" to "extremely". VAS scores were obtained at the same time points as the nicotine plasma concentrations sampling time points, i.e. before (0), 2, 4, 8, 16, 24 and 30 minutes after each product was administered:

- overall "product strength" (head rush, "buzz", "hit", feeling alert)
- craving intensity
- increased salivation
- burning sensation in the mouth and/or throat

For flow chart please see Appendix 6.

10.4.2 Appropriateness of Measurements - Choice of Primary Outcome variable

10.4.2.1 Sampling Procedures and analysis of used snus

The nicotine content per portion of used and unused snus, respectively, was estimated using a method modified from the Coresta Recommended Method, No 62. The mean $\pm$ SD extracted dose of nicotine from one portion of snus, was calculated. Nicotine was extracted from the snus using sodium hydroxide and methyl-tert-butyl ether containing quinoline as an internal standard. The nicotine present in the extract is determined by using a gas chromatograph equipped with a flame ionization detector.

10.4.2.2 Nicotine Concentration Measurements in plasma

Frozen plasma samples collected for nicotine determinations were shipped to a certified contract laboratory. The analysis of nicotine in the plasma samples was performed by LC-MS/MS at ABS Laboratories Ltd, UK.

To quantify nicotine a multilevel calibration at seven concentrations was performed. The calibration line was fitted by means of a power curve fitting regression model using the equation $y=ax^b$. The samples were assayed once. If the sample shows concentrations considered by the Study Director to be outside those expected the sample was re-assayed. If the repeat assay gave a result greater than ±10% of the first result a third analysis was performed, subject to the availability of sample. The precision of the method above the 0.7

ng/ml level of nicotine is better than 12% C.V. and above 4 ng/ml better than 6% C.V. The level of quantification is 0.5 ng/ml.

An analysis was made on cotinine which is the main nicotine metabolite, to investigate if it is formed to a higher extent from the products with higher nicotine content. This would be the case if a higher fraction of nicotine was swallowed. Cotinine plasma concentrations in smokers at steady state are about 100 times those of nicotine.

The primary effectiveness measure for evaluation was the change in subjective effects such as head rush and craving, assessed by a patient-reported outcome (PRO), the visual analogue scale (VAS). The VAS is a well-known and validated assessment instrument for evaluation of subjective effects [West R & Ussher M 2010]. It may reveal beneficial treatment effects, from the patient's perspective, not captured by objective monitoring. The VAS is sufficiently simple and robust to be suitable for research. The change in subjective effects was defined as the difference in the VAS score from start to end of each drug administration (=over 30 minutes after each trial product was administered).

10.5 Primary Efficacy Variables

Noncompartmental Analysis using WinNonlin computer program (Siphar Corp., USA) was used for all pharmacokinetic calculations.

- Extracted dose
- Nicotine plasma concentrations are determined at preset time points, before (0), 2, 4, 8, 16, 24, 30, 45, 60, 90 minutes and 2, 4 and 6 hours after administration.
- C_{max} and T_{max}
- AUC_{inf}

10.6 Primary Safety Variables

Primary safety variables were increased salivation and burning sensation in the mouth and/or throat, respectively, assessed by the visual analogue scale (VAS).

All other safety assessments were standard, i.e., widely used and generally recognized as reliable, accurate, and relevant.

10.7 Data quality assurance

Electronic case report forms (eCRF) in palm top computers were used for recording of subjective symptoms (head rush and craving, salivation and burning sensation in the mouth and/or throat). Investigator meetings and training sessions were conducted at the investigation site in this single centre study to ensure the collection of accurate, consistent, complete, and reliable data. Instruction manuals were used.

Monitoring by CRO personnel included 100% source data verification (SDV). Independent external audits of the clinical center plus the CRO files were conducted. Audit certificates were provided. Please see Appendix.

10.8 Statistical Methods Planned in the Protocol and Determination of Sample Size

10.8.1 Statistical and Analytical Plans

Only descriptive statistics were used. Due to the different characteristics of the five nicotine products compared, a common bioequivalence test was not feasible and a strict statistical hypothesis and power analysis were not possible. Results are summarized as mean ± SD values and 90% confidence interval (CI). Differences between groups in continuous outcome measures were analyzed using unpaired t-tests.

All baseline and demographic data is presented by using descriptive statistics and frequency tables, divided by treatment group. Laboratory data was described as changes from baseline.

All adverse events were analyzed as maximum intensity during treatment. A separate analysis was done on those adverse events, which were related to study drug.

10.8.2 Determination of Sample Size

The present study should assess the comparison of Swedish snus to one pharmaceutical NRT product reference, 6 mg of Nicorette sublingual nicotine tablets. The sample size calculation is based on the comparison between 6 mg of Nicorette sublingual nicotine tablets and

Swedish Snus (16 mg) without loss of generality, for simplicity and without adjusting for Type 1 error due to multiple comparisons (Chow, Shao and Wang 2003).

Linear pharmacokinetics has been shown for buccal administration of nicotine in the dose interval 1-4 mg (Molander L and Lunell E 1999).

A previous single-dose study (Lunell E and Curvall M 2011), showing an average nicotine extraction from the 8 mg Swedish Snus to be 2.18±0.92 mg made a calculation of sample size possible. Under the assumption of a complete disintegration and extraction of the 6 mg of Nicorette sublingual nicotine tablets versus the 2.18±0.92 mg nicotine, extraction leveling off above the 8mg strength for the Swedish Snus and a standard deviation of 5.0 the estimated sample size is 16 with a power of 80% and alpha=0.05.

The randomization was performed using Latin Squares approach.

10.8.3 Pharmacokinetic assessments

Plasma samples drawn at regular intervals for up to 6 hours after dose administration were analyzed for nicotine. Plasma concentration-time curves of nicotine and pharmacokinetic variables such as C_{max}, T_{max} and AUC_{inf} of one single dose of various nicotine formulations are standard objective methods for evaluation of NRT products. Descriptive statistics (Mean±SD) were used for comparison of the five products. C_{max}, T_{max}, and AUC_{inf}, for 1 g portion SS (16 mg and 8 mg nicotine, respectively), 0.5 g portion SS (8 mg nicotine) and 2 x 1 g portion SS (2x8 mg nicotine) in comparison with 6 mg of Nicorette® Microtab, were calculated. The plasma concentration-time curves were presented separately for each individual and the corresponding data were tabulated.

Comparisons between the different Swedish Portion Snus preparations and versus 6 mg of Nicorette® Microtab regarding the C_{max}, T_{max}, and AUC_{inf} were made using Wilcoxon Rank Sum test.

The calculations were based on a standard error estimated from a four period crossover analysis of variance model accounting for the following sources of variation: Sequence, subject (sequence), period, treatment and carry-over (only included in model if found significant).

The AUC_{inf} of each of the Snus products was used for demonstration of the relative dose absorbed into the systemic blood circulation. The geometric mean and 90% confidence

interval of the AUC_{inf} ratio of Snus products were pair-wise estimated. Assessment of dose linearity was based on pooled individual data of four different snus products (B, C, D, E).

The VAS scores of subjective effects of Swedish Portion Snus were compared with 6 mg of Nicorette® Microtab.

10.9 Protocol Deviations

10.9.1 Drop-outs

No patient dropped out from the study.

10.9.2 Invalid Baseline Nicotine Plasma Recordings

There were two subjects, No 2 and No17, with baseline nicotine plasma levels exceeding 4 ng/ml, indicating protocol violation (cheating). These subjects were therefore excluded from the analysis of the results.

10.9.3 Lost data from Patient operated electronic Case Record Forms

There were a few VAS data lost from the electronic CRF (eCRF) recorded on the palm top computer, due to technical problems with battery function in the units, causing the attached computer program to break down and data were lost.

Protocol deviations are described Appendix 16.2.2. Individual protocol deviations are broken down by patient and centre.

Date: 2013-06-17

RESULTS

11 EFFICACY EVALUATION

11.1 Characteristics of study subjects

Individual baseline demographic and clinical characteristics are given in Table 1. Eighteen randomised subjects successfully completed all sessions. Due to protocol violation (cheating) by subjects No 2 and No. 17 sixteen (16) subjects were eligible for primary efficacy analysis of all five treatments.

(b) (6)

Table 1. Characteristics of study subjects. N=18.

Pat No	Initials	Sex	Age	Height (cm)	Weight (kg)	No of portions /day	Since (yrs)	Brand	Ex-smoker
1	(b)	M	19	174	72	13	4	Swedsnus	No
2*	(b)	M	43	183	90	24	29	Granit	Yes
3	(b)	M	20	173	80	12	3	General	Yes
4	(b)	M	46	189	99	18	25	General	Yes
5	(b)	M	49	176	88	42	15	General	Yes
6	(b)	M	33	185	110	10	9	Gbg Rapé	Yes
7	(b)	M	39	185	129	30	25	Granit	No
8	(b)	M	21	184	90	10	6	General	No
9	(b)	M	23	170	57	12	6	Gbg Rapé	No
10	(b)	M	19	185	81	6	2	General	Yes
11	(b)	M	58	184	99	30	25	Kaliber	No
12	(b)	F	50	164	56	10	10	Catch liq	Yes
13	(b)	M	52	178	86	7	20	Probe	Yes
14	(b)	M	47	180	90	11	30	General	Yes
15	(b)	F	45	181	70	11	10	Catch White	Yes
16	(b)	M	19	170	54	18	5	Kaliber	No
17*	(b)	M	20	190	80	10	2	General	No
18	(b)	M	19	185	92	14	5	General	No
Mean		16 M /2 F	34. 6	179. 8	84. 6	16	12. 8		
SD			14. 3	7. 2	19. 0	9.8	10. 0		
Min			19	164	54	6	2		
Max			58	190	129	42	30		

* Two subjects, No 2 and No 17, with baseline nicotine plasma levels exceeding 4 ng/ml were excluded from pharmacokinetic analysis.

Date: 2013-06-17

11.2 Efficacy evaluation – Pharmacokinetics

11.2.1 Extracted amount of Nicotine and Tablet disintegration time

Ten pouches of unused Snus B, PSWM 0.5 g (16 mg nicotine/g) contained a mean amount of 7.31 mg nicotine/portion. The used pouches of Snus B, analyzed for nicotine content showed a mean (±SD) residual amount of 5.41±0.82 mg (range 3.46 - 6.54mg). The mean (±SD) extracted amount of nicotine from Snus B thus was estimated at 1.90±0.82 mg (range 0.77 – 3.85 mg) nicotine/portion.

Ten pouches of unused Snus C, PSWL 1g (8 mg nicotine /g) contained a mean (±SD) amount of 7.72 mg nicotine/portion. The used pouches of Snus C, analyzed for nicotine content showed a mean (±SD) residual amount of 6.16±0.95 mg (range 3.31- 6.92 mg). The mean (±SD) extracted amount of nicotine from Snus C, thus was estimated at 1.56 ±0.95 mg (range 0.80 – 4.41 mg) nicotine/portion.

Ten pouches of unused Snus D, PSWL 1g (16 mg nicotine /g) contained a mean (±SD) amount of 15.7 mg nicotine/portion. The used pouches of Snus D, analyzed for nicotine content showed a mean (±SD) residual amount of 12.7±1.65 mg (range 7.0 - 14.1 mg). The mean (±SD) extracted amount of nicotine from Snus D, PSWL 1 g (16 mg nicotine /g), thus was estimated at 3.0 ±1.65 mg (range 1.6 – 8.8 mg) nicotine/portion.

Twenty pouches of unused Snus E, PSWL (8 mg nicotine /g) 2x1 g, contained a mean (±SD) amount of 15.4 mg nicotine/2 portions. The used pair of pouches of Snus E, analyzed for nicotine content showed a mean (±SD) residual amount of 12.4±1.35 mg (range 9.41- 13.8 mg). The mean (±SD) extracted amount of nicotine from Snus E thus was estimated at 3.0 ±1.35 mg (range 1.6 – 6.0 mg) nicotine/portion.

Table 2. Nicotine extraction from Snus products B – E. N=18.

	Snus B **PSWM 0.5 g** **(16 mg nicotine/g)**	**Snus C** **PSWL 1 g** **(8 mg nicotine /g)**	**Snus D** **PSWL 1 g** **(16 mg nicotine /g)**	**Snus E** **PSWL 2x1 g** **(8 mg nicotine /g)**
Mean (mg)	1.90	1.56	3.0	3.0
SD	0.82	0.95	1.65	1.35
Min	0.77	0.80	1.6	1.6
Max	3.85	4.41	8.8	6.0

The mean (±SD) extracted amount of nicotine from Snus B, C, D, E is demonstrated in Table 2. Individual values of the In-vivo extracted dose per kg body weight are given in Section 15.1.

Mean (±SD) *in-vivo* disintegration time of 3 tablets (=6mg) of Nicorette® Microtab was 58.4 ±12.3 minutes (range 30-80 minutes). This was longer than expected and may have been due to formation of a larger aggregate in the oral cavity of the subjects. Subjects were instructed to keep the tablets still and not to suck on or chew them. Individual values are given in Section 15.1.

11.2.2 Nicotine Plasma Concentration-time Profiles.

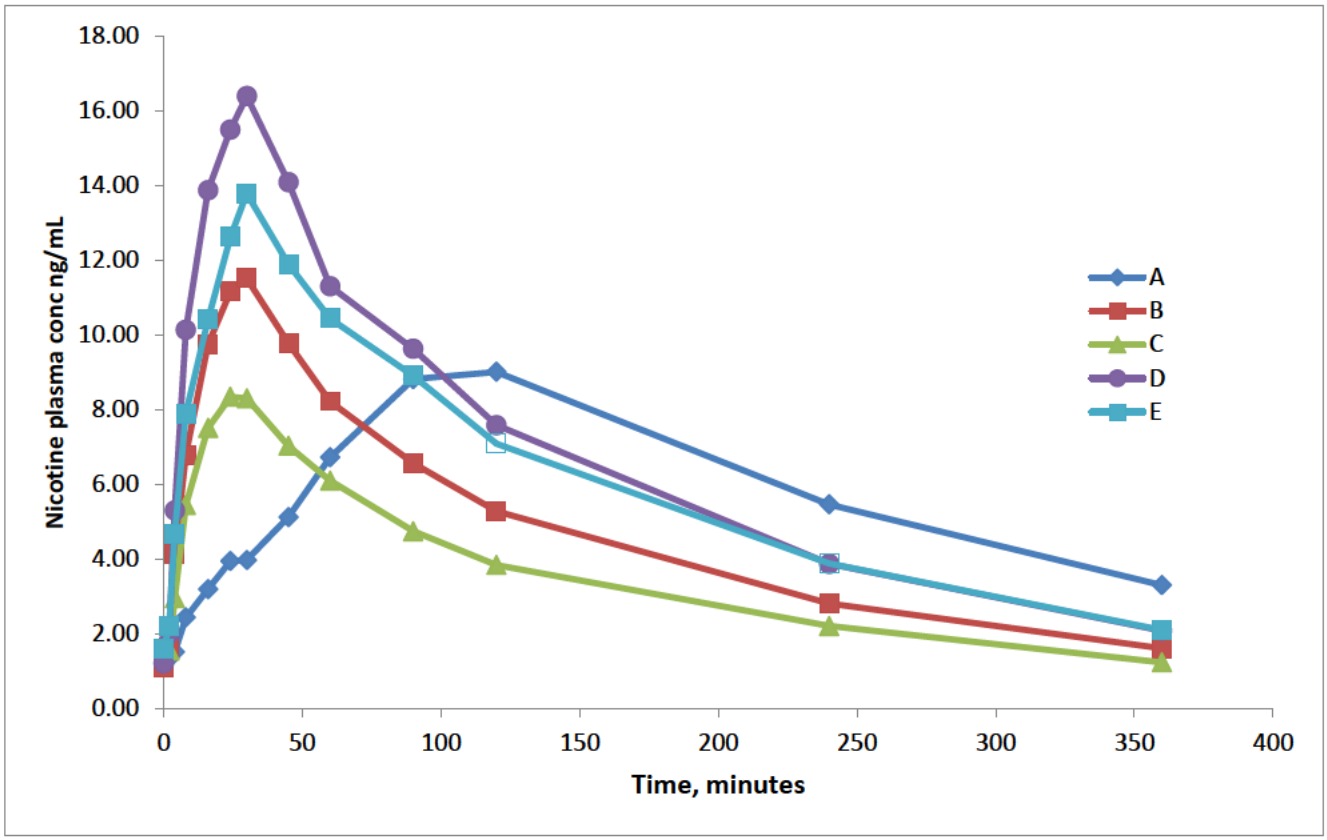
The plasma concentration-time curves appeared smooth with no indication of non-compliance. Please see Figure 1.

For individual nicotine plasma concentration – time curves please see Section 16.2. For individual nicotine plasma concentration values please see Section 16.3.

The rise of the nicotine plasma concentration was faster for the Snus products compared to the Nicorette® Microtab. The median T_{max} was shorter, 24-30 minutes, for all four strength of SS compared to compared to the nicotine tablets. The C_{max} of the SS products B, D and E were higher than the C_{max} for the nicotine tablets. Please see nicotine plasma concentration – time curves in Figure 1.

Three tablets (=6mg) of Nicorette® Microtab produced an AUC_{inf} in the same order of magnitude as the nicotine products containing the high dose, 16mg nicotine, around 3000 ng*min/mL, however with a considerably later T_{max}, compared to the Snus products.

Date: 2013-06-17
Figure 1. Nicotine plasma concentration-time curves. N=16



Legend:

A= 6 mg dose of Nicorette® Microtab sublingual nicotine tablets (=3 tablets).

B= Swedish Portion Snus PSWM 0.5 g (16 mg nicotine/g)

C=Swedish Portion Snus PSWL 1 g (8 mg nicotine /g)

D= Swedish Portion Snus PSWL 1 g (16 mg nicotine /g)

E= Swedish Portion Snus PSWL 2x1 g (8 mg nicotine /g)

11.2.3 Maximal Nicotine Plasma Concentration (C_{max})

The mean (± SD) C_{max} achieved after administration of 6 mg of Nicorette® Microtab was 10.40±4.65 ng/mL (range 11.47-17.93 ng/mL).

The C_{max} after administration of the Snus B, PSWM 0.5 g (16 mg nicotine/g) was 12.22 ± 4.23 ng /mL (range 7.81 – 22.14 ng/mL). This corresponds to 114% of the C_{max} after administration of 6 mg of Nicorette® Microtab.

The C_{max} after administration of the Snus C, PSWL 1.0 g (8 mg nicotine /g) was 9.03 ± 3.66 ng /mL (range 4.30 – 17.73 ng/mL). This corresponds to 85% of the Cmax after administration of 6 mg of Nicorette® Microtab.

The C_{max} after administration of the Snus D, PSWL 1.0 g (16 mg nicotine /g) was 17.66± 6.78 ng/mL (range 9.05 – 29.81 ng/mL). This corresponds to 167% of the Cmax after administration of 6 mg of Nicorette® Microtab.

The C_{max} after administration of the Snus E, PSWL 2x1g (8 mg nicotine /g) was 14.41 ± 5.45 ng/mL (range 8.12 – 27.27 ng/mL). This corresponds to 136% of the Cmax after administration of 6 mg of Nicorette® Microtab.

The measured mean maximal plasma nicotine concentrations (C_{max}) are shown in Table 3. The individual and mean (± SD) measured C_{max} values are displayed in Section 15.5.

Table 3. C_{max} values for all treatments. N=16.

C_{max} (ng/mL)	**Microtab (A)**	**Snus B PSWM 0.5 g (16 mg nicotine/g)**	**Snus C PSWL 1 g (8 mg nicotine /g)**	**Snus D PSWL 1 g (16 mg nicotine /g)**	**Snus E PSWL 2x1 g (8 mg nicotine /g)**
Mean	10.40	12.22	9.03	17.66	14.41
SD	4.65	4.23	3.66	6.78	5.45
Min	11.47	7.81	4.30	9.05	8.12
Max	17.93	22.14	17.73	29.81	27.27

Two subjects, No 2 and No17, with baseline nicotine plasma levels exceeding 4 ng/ml were excluded from pharmacokinetic analysis.

11.2.4 Time to Maximal Nicotine Plasma Concentration (T_{max})

The median T_{max} following administration of the Snus B, PSWM 0.5 g (16 mg nicotine/g), was 27 minutes (range 8-45 minutes).

The median T_{max} after administration of the Snus C, PSWL 1 g (8 mg nicotine /g), was the shortest of all products, 24 minutes (range 16-90 minutes).

The median T_{max} after administration of the Snus D, PSWL 1 g (16 mg nicotine /g), and Snus E, PSWL 2x1 g (8 mg nicotine /g), was 30 minutes (range 16-90 minutes), respectively.

For comparison, the median time to maximal plasma concentration (T_{max}) achieved after administration of 6 mg of Nicorette® Microtab was 105 minutes (range 24-240 minutes).

The median T_{max} values are shown in Table 3. The individual and median T_{max} values are displayed in Section 15.6.

Table 3. Median T_{max} values for all treatments. N=16.

T_{max} (minutes)	**A. Nicorette® Microtab 6 mg**	**Snus B PSWM 0.5 g (16 mg nicotine/g)**	**Snus C PSWL 1 g (8 mg nicotine /g)**	**Snus D PSWL 1 g (16 mg nicotine /g)**	**Snus E PSWL 2x1 g (8 mg nicotine /g)**
Median	105	27	24	30	30
Min	24	8	16	16	16
Max	240	45	90	90	90

11.2.5 Area under the Nicotine Plasma Concentration-Time Curve (AUC_{inf})

The mean (± SD) extent of nicotine bioavailability, i.e. the area under the nicotine plasma concentration-time curve, AUC_{inf} after administration of 6 mg of Nicorette® Microtab was 2976.33 ± 1491.84 ng∗min/mL.

The AUC_{inf} after administration of the Snus B, PSWM 0.5 g (16 mg nicotine/g), was 2032.65± 724.53 ng*min/mL. This corresponds to 68.3% of the AUC_{inf} after administration of 6 mg of Nicorette® Microtab.

The AUC_{inf} after administration of the Snus C, PSWL 1 g (8 mg nicotine /g), was 1484.35 ± 605.30 ng*min/mL. This corresponds to 50.1% of the AUC_{inf} of 6 mg of Nicorette® Microtab.

The AUC_{inf} after administration of the Snus D, PSWL 1 g (16 mg nicotine /g), was 2792.31± 1015.33 ng*min/mL. This corresponds to 93.8% of the AUC_{inf} of 6 mg of Nicorette® Microtab.

The AUC_{inf} after administration of the Snus E, PSWL 2x1 g (8 mg nicotine /g), was 2639.71± 879.70 ng*min/mL. This corresponds to 88.7% of the AUC_{inf} of 6 mg of Nicorette® Microtab.

The calculated mean AUC_{inf} values are shown in Table 5. Individual and mean (±SD) AUC_{inf} values are displayed in Section 15.7.

Table 5. AUC_{inf} values for all treatments. N=16.

AUC_{inf}	A. Nicorette® Microtab 6 mg	Snus B PSWM 0.5 g (16 mg nicotine/g)	Snus C PSWL 1 g (8 mg nicotine /g)	Snus D PSWL 1 g (16 mg nicotine /g)	Snus E PSWL 2x1 g (8 mg nicotine /g)
Mean	2976.33	2032.65	1484.35	2792.31	2639.71
SD	1491.84	724.53	605.30	1015.33	879.70
Min	447.21	1108.70	824.71	1412.46	1369.23
Max	5754.70	3671.95	2797.74	4749.22	4663.79

Two subjects, No 2 and No17, with baseline nicotine plasma levels exceeding 4ng/ml were excluded from pharmacokinetic analysis.

11.2.6 Bioequivalence (AUC_{inf} and C_{max} ratios)

Primary objective was to compare AUC_{inf} after administration of a single dose of 6 mg Nicorette sublingual nicotine tablets (Nicorette Microtab®) to that of one single dose of SS containing 16 mg nicotine (Snus D). The geometric mean and 90% confidence interval of the AUC_{inf} ratio of the tablet and the Snus D were estimated at 0.962 (0.758 - 1.221). From these results it may

be concluded that the two formulations were not bioequivalent according to strict criteria with respect to AUC_{inf}, however very close to bioequivalence (90% CI within 0.80-1.25).

Secondary objective was to compare AUC_{inf} of Snus D (16 mg nicotine) to AUC_{inf} of Snus E (2x8mg nicotine). The geometric mean and 90% confidence interval of the AUC_{inf} ratio of the Snus D and Snus E were estimated at 1.049 (0.942 - 1.168). From these results it may be concluded that the two formulations were bioequivalent with respect to AUC_{inf}.

Secondary objective was also to compare AUC_{inf} of 0.5 g portion SS containing 8 mg nicotine (Snus B) to AUC_{inf} of 1 g portion SS containing 8 mg nicotine (Snus C). The geometric mean and 90% confidence interval of the AUC ratio of the Snus B and Snus C were estimated at 1.393 (1.273 - 1.524). Portion SS 0.5 g containing 8 mg nicotine (Snus B) thus displayed a higher bioavalilability of nicotine compared to 1 g portion SS containing 8 mg nicotine (Snus C) measured as AUC_{inf}.

Table 6. Analysis of bioequivalence and dose proportionality for AUC_{inf} versus bodyweight. N=16.

Analysis of Bioequivalence (c)	Log mean ratio (90% CI) [a]	Mean ratio (90% CI) [b]
A vs D: ln_AUC	-0.039 (-0.278 - 0.200)	0.962 (0.758 - 1.221)
A vs D: ln_Cmax	-0.589 (-0.843 - -0.335)	0.555 (0.430 - 0.715)
B vs C: ln_AUC	0.331 (0.242 - 0.421)	1.393 (1.273 - 1.524)
B vs C: ln_Cmax	0.323 (0.202 - 0.445)	1.382 (1.223 - 1.560)
D vs E: ln_AUC	0.048 (-0.060 - 0.155)	1.049 (0.942 - 1.168)
D vs E: ln_Cmax	0.198 (0.037 - 0.358)	1.218 (1.037 - 1.431)
Dose proportionality [d]	DF=12.9	0.692 (0.578 - 0.805)
[a] Log scale, i.e. Ln(A) - Ln(B). [b] Backtransformed to original scale, i.e. A/B. [c] Students t-test on the paired differences [d] From power model: ln AUC = $\alpha + \beta$*ln dose. Confidence interval for β is given. DF: Degrees of Freedom.		

As expected the two formulations A and D were not bioequivalent with respect to C_{max}, the sublingual tablet (A) showing significantly lower C_{max} compared to snus D, geometric mean and 90% CI of the ratio being 0.555 (0.430 - 0.715).

Neither were the two formulations B and C bioequivalent with respect to C_{max}, the Snus B thus showing a higher peak concentration of nicotine compared to Snus C, despite containing the same amount of nicotine, 8 mg nicotine. The geometric mean and 90 % CI of the C_{max} ratio were 1.382 (1.223 - 1.560).

When Snus D (16 mg nicotine) was compared to AUC_{inf} of Snus E (2x8mg nicotine) the geometric mean and 90% CI of the C_{max} ratio were 1.218 (1.037 - 1.431). There was thus a faster absorption of nicotine from Snus D than from Snus E despite the fact that the two formulations were bioequivalent with respect to AUC_{inf}.

11.2.7 Dose proportionality of AUC_{inf} (linear pharmacokinetics). Relationship between In-vivo extracted dose and area under the plasma concentration – Time curve (AUC).

Assessment of dose proportionality (linear pharmacokinetics) was based on pooled data on the four different snus administrations (B, C, D, E). The subjects were quite widely distributed with respect to extraction, from 10 to 57% of the nicotine content in each pouch. Further, their body weight showed relatively large distribution, from 54 to 129 kg. Consequently the *In-vivo* extracted dose versus kg body weight showed wide distribution. See figure 15.8.3. However, the most interesting issue was whether there was any strong relationship between extracted dose and nicotine uptake into the blood circulation, i.e. $AUC_{inf.}$ AUC_{inf} is dependent on body weight, since it corresponds to the distribution of each dose to the body circulation and tissues. The dose proportionality was examined by comparison of the relationship between the AUC_{inf} and the extracted dose per kg body weight. The plot showed a fairly linear relationship, and even better linearity when plotted in a logarithmic X-Y diagram, see Section 15.8. A good linearity was also obtained when AUC_{inf} was plotted against extracted dose/Body Surface Area (BSA) (R^2=0.6632), see Figure 2.

As is evident from the 90% confidence interval for the β coefficient in the power model, dose proportionality could not be concluded. However, the entire 90% confidence interval falls below 1 which is indicative of a relationship between dose and AUC on the LOG scale (for each one unit increase in LOG dose, LOG AUC increases with 0.692 units). The p-value for the β coefficient in the power model is p<0.0001. See Table 6.

The inter-individual extraction showed quite high variability, from 10 to 57%. However, the intra-individual variability between the four different snus products (B, C, D, E) was rather small. Each subject showed a quite high consistency with respect to extraction, 9 subjects being ‘low extractors’ showing a mean 12-18% extraction and 9 subjects being ‘high

extractors', showing a 19-51% extraction. See table 15.1.e. Individual factors such as salivation and discreet oral movements may separate the two groups, however were not possible to measure.

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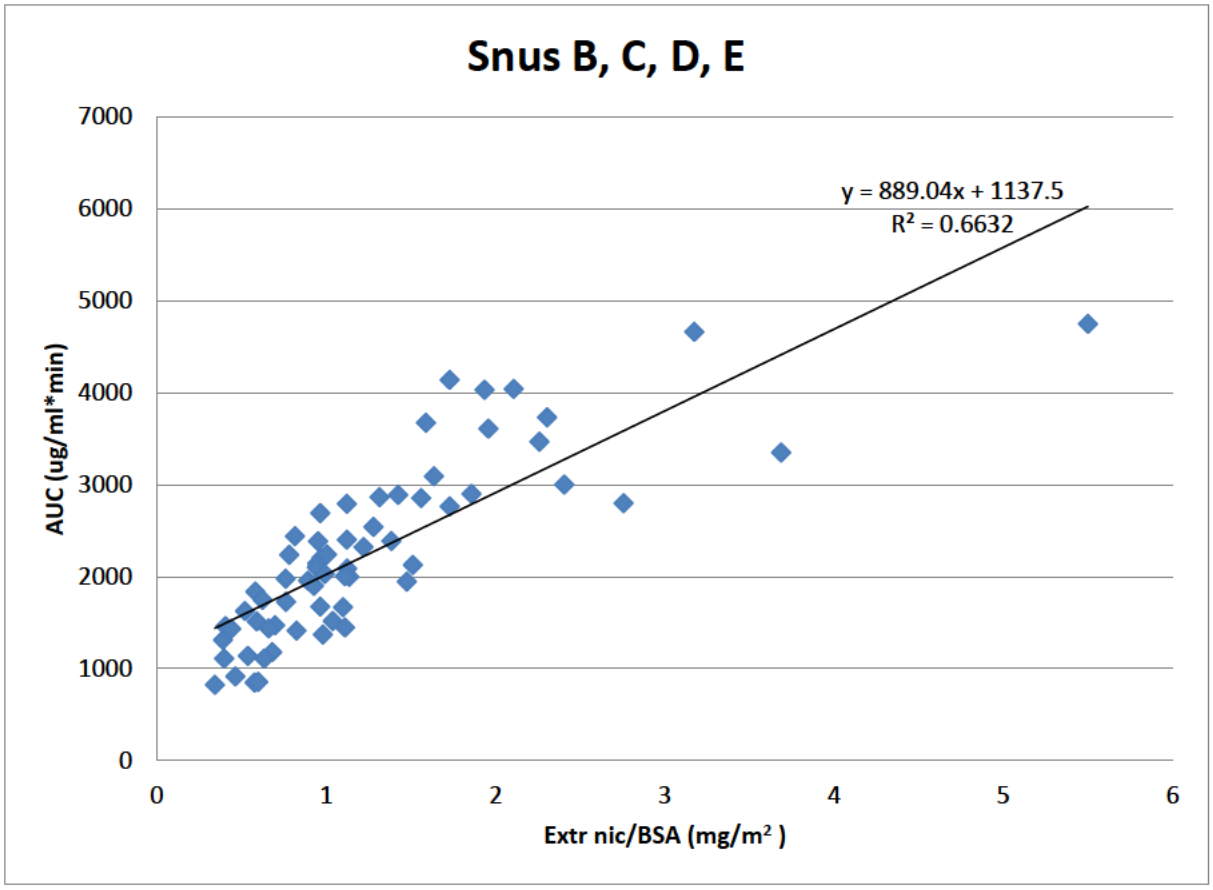
Figure 2. Composed diagram of AUC versus mg extracted nicotine/body surface area (m^2) for all Snus products.



11.2.8 Subjective effects - Head Rush

The subjective ratings of "Head Rush" on the Visual Analogue Scale (VAS) were in range 1.00 – 2.21 in the morning after one night's abstinence before any trial product had been used. This was true for all sessions. After 30 minutes the ratings had increased significantly for all products tested.

The values of these ratings increased rapidly to reach a maximum at 8-24 minutes. The mean 8 minute values for "Head Rush" were 2.81, 3.38, 3.87, 3.69, for Snus B, C, D, E, respectively, compared to 2.73 for 6 mg of Nicorette® Microtab at 30 minutes. The mean values of these ratings at 30 minutes were 3.06, 2.75, 3.53, and 3.88 for Snus B, C, D, E, respectively, compared to 3.08 for 6 mg of Nicorette® Microtab. The mean ratings of "Head Rush" on the Visual Analogue Scale (VAS) are shown in Figure 3 and Table 5. The individual scores are shown in Section 17.

Table 5. Mean Head rush VAS score, including maximum value (bold). N=16

Time (Minutes)	A - Tablet	Snus- B	Snus - C	Snus - D	Snus - E
0	2.21	1	1.5	1.73	1.47
2	2	2.63	1.94	2.2	2
4	2.73	2.81	2.94	2.93	2.75
8	2.73	2.81	**3.38**	**3.87**	3.69
16	3	2.93	3.31	3.73	**3.88**
24	3.07	**3.2**	3.19	3.73	3.81
30	**3.08**	3.06	2.75	3.53	3.88

The statistical analysis showed significantly higher head rush values for E compared to B at time point 8 minutes and 16 minutes, p-values 0.0439 and 0.0225, respectively. For the comparison between E and C there were significantly higher values for E at time point 30 minutes, p-value 0.0232. Statistical analyses are shown in Section 18.

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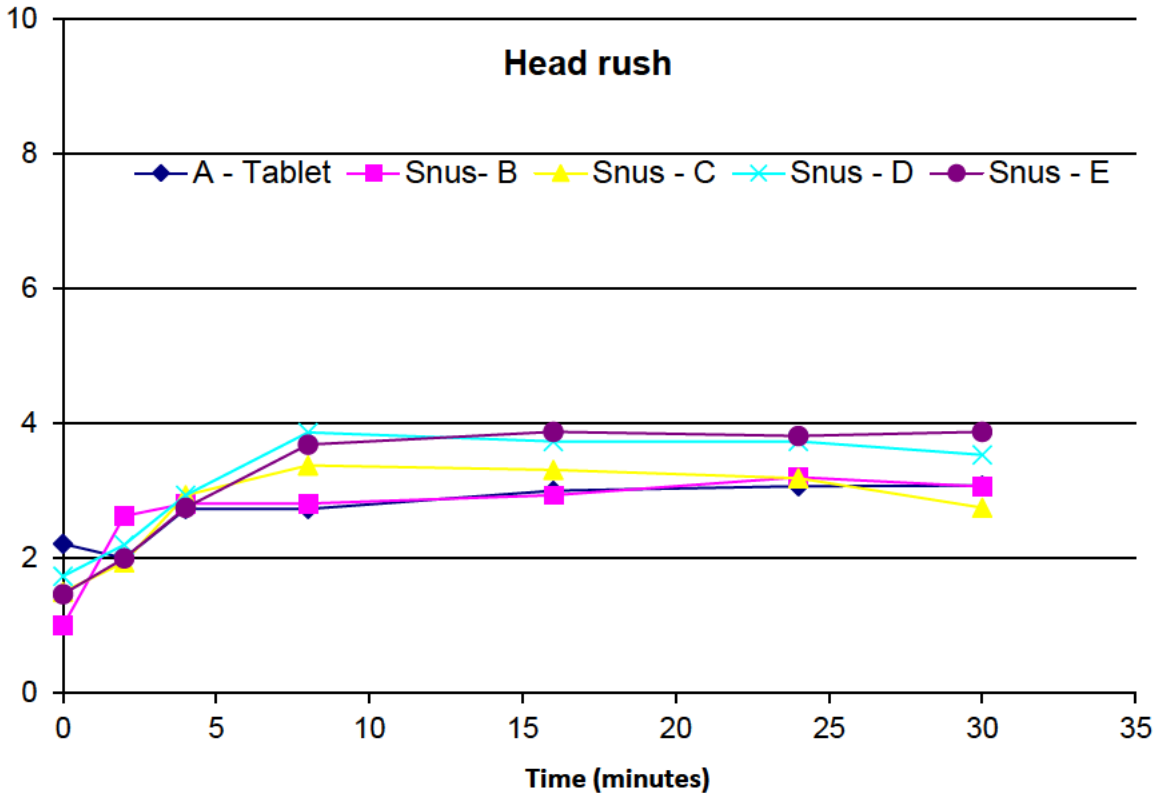
Figure 3. Mean Subjective VAS ratings of "head rush". N=16



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11.2.9 Craving

The subjective ratings of craving on the Visual Analogue Scale (VAS) were highest in the morning after one night's abstinence before any trial product had been used. The mean baseline values of these ratings were very similar for all sessions, range 4.21 – 4.93. After 30 minutes the ratings had decreased significantly for all products tested. The mean values of these ratings after 30 minutes were 1.75, 1.63, 1.47, 1.00, for Snus B, C, D, E, respectively, compared to 2.38 for 6 mg of Nicorette® Microtab. There was no significant difference between the various Snus products, and 6 mg of Nicorette® Microtab for craving.

The mean ratings of craving on the Visual Analogue Scale (VAS) are shown in Figure 4 and Table 6. The individual scores are shown in Section 17.

Table 6. Mean Craving VAS score, including minimum value (bold). N=16.

Time (Minutes)	A - Microtab	Snus- B	Snus - C	Snus - D	Snus – E
0	4.21	4.38	4.81	4.93	4.33
2	4.14	3.06	3.94	3.67	4.13
4	3.87	2.88	2.94	3.20	3.38
8	3.20	2.63	2.13	2.13	2.19
16	3.21	2.33	1.94	1.80	1.19
24	2.67	1.80	2.00	**1.40**	1.13
30	**2.38**	**1.75**	**1.63**	1.47	**1.00**

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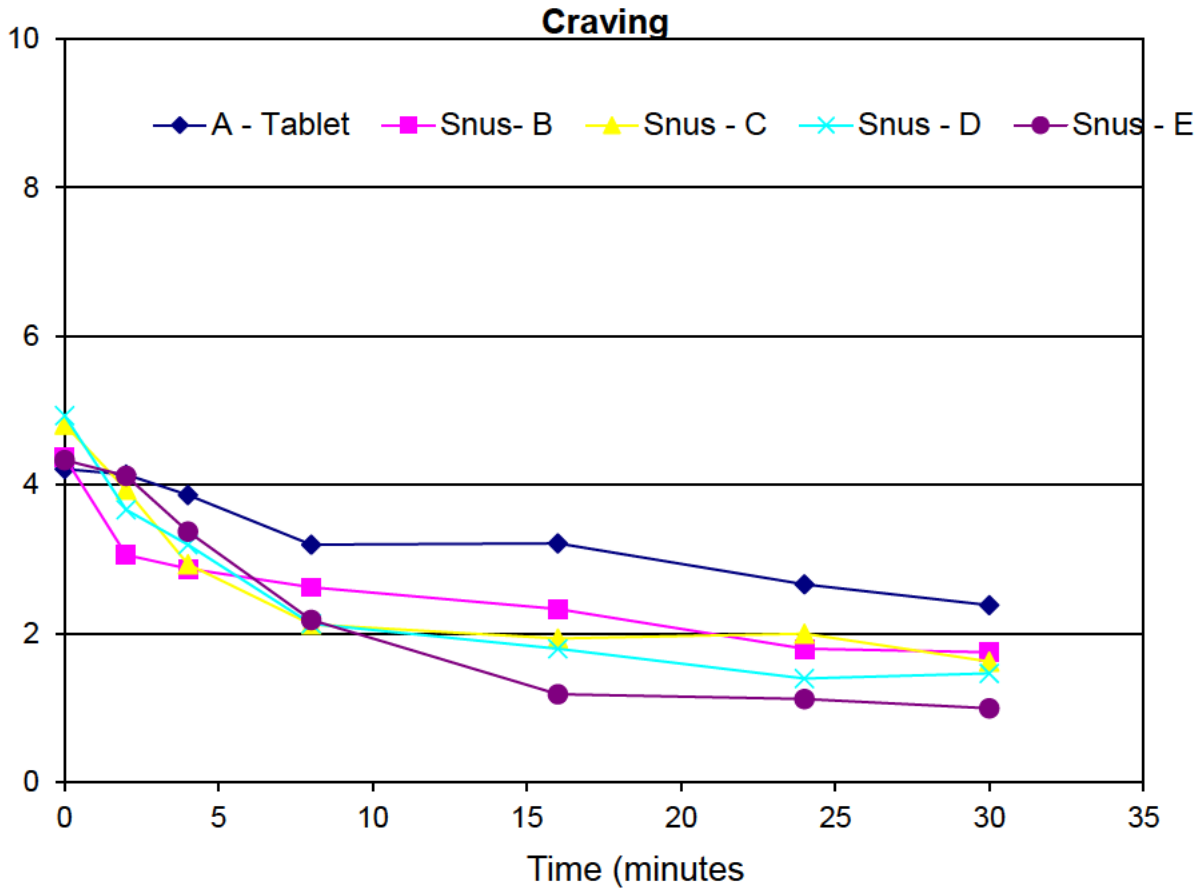
Figure 4. Mean subjective VAS ratings of craving. N=16.



12 SAFETY EVALUATION

12.1 Tolerability

Each subject's rating of salivation and burning sensation in the mouth/throat, respectively, indicated on a Visual Analogue Scale (VAS) anchored with "not at all" to "extremely" was obtained at the time points 0, 10, 20 and 30 minutes after 8 and 16 mg Swedish Portion Snus and 6 mg of Nicorette® Microtab were administered. It should be noted that all subjects were regular Snus users and no one had tried nicotine sublingual tablets previously.

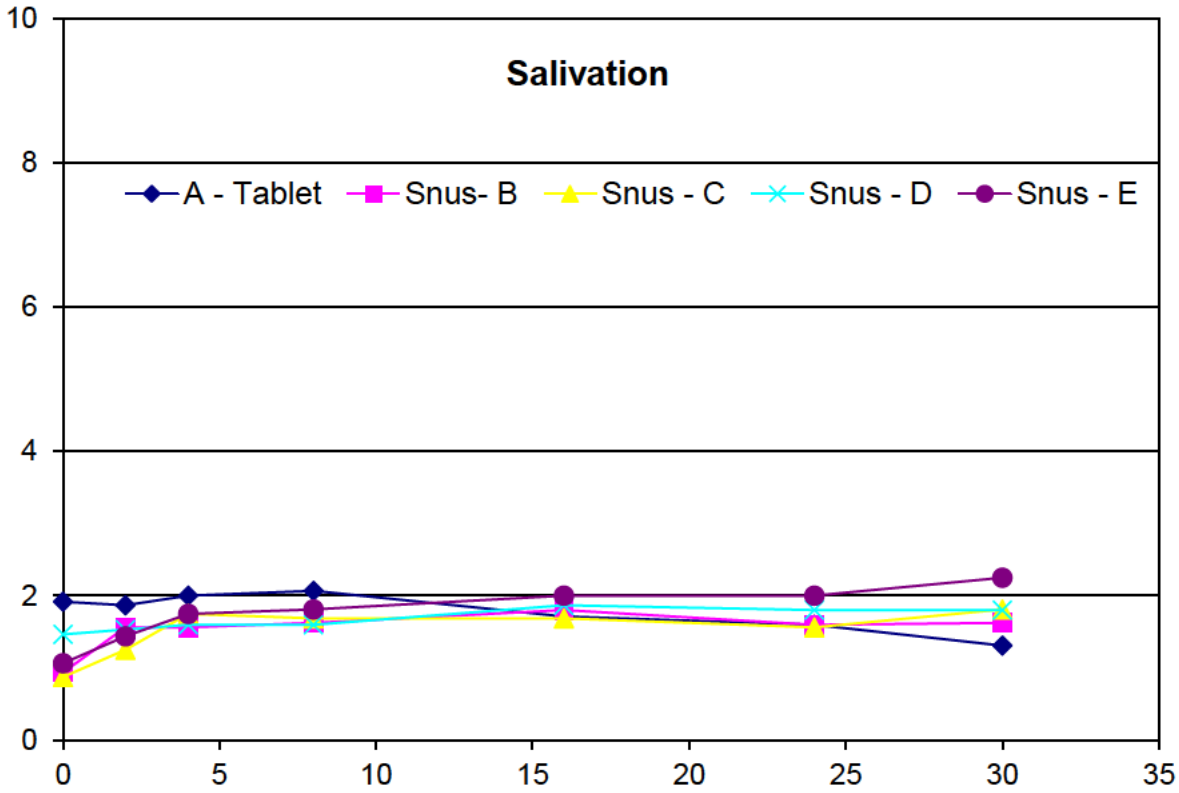
12.1.1 Salivation

The mean salivation scores were very similar through all Snus sessions as well as for 6 mg of Nicorette® Microtab. The mean results for rating of salivation are shown in Figure 5 and Table 7. The individual ratings are tabulated in and Section 17.

Table 7. Mean Salivation VAS score, including maximum value (bold).

Time (Minutes)	A - Tablet	Snus- B	Snus - C	Snus - D	Snus - E
0	1.92	0.94	0.88	1.47	1.07
2	1.87	1.56	1.25	1.53	1.44
4	2	1.56	1.75	1.60	1.75
8	**2.07**	1.63	1.69	1.60	1.81
16	1.71	**1.80**	1.69	**1.87**	2
24	1.60	1.60	1.56	1.80	2
30	1.31	1.63	**1.81**	1.80	**2.25**

Figure 5. Mean Subjective VAS ratings of salivation. N=16.



12.1.2 Mouth/throat burn

The mean score for burning sensation were very similar through all Snus sessions as well as for 6 mg of Nicorette® Microtab.

The mean results rating of a burning sensation are shown in Figure 6 and Table 8. The individual ratings are tabulated in Section 17.

Table 8. Mean Mouth/throat burn VAS score, including maximum value (bold). N=16.

Time (Minutes)	A - Microtab	Snus- B	Snus - C	Snus - D	Snus - E
0	0.33	0.75	0.94	1.40	1.13
2	0.60	1.06	1.07	1.60	0.88
4	0.73	1.19	1.13	**1.67**	1.31
8	0.67	1.19	**1.56**	1.40	1.56
16	**1.00**	1.20	1.31	1.53	1.19
24	0.93	**1.33**	1.19	1.60	1.50
30	0.85	1.31	1.31	1.60	**1.75**

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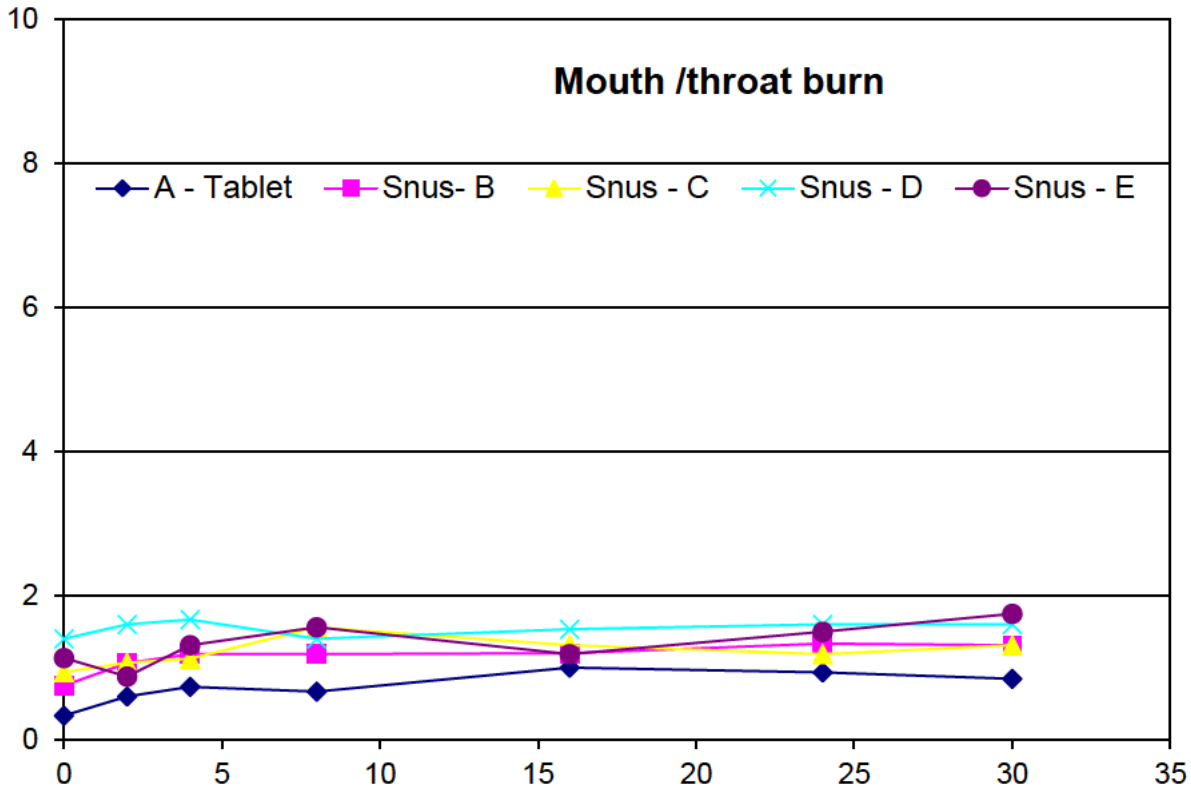
Figure 6. Mean Subjective VAS ratings of mouth/throat burn. N=16.



12.2 Adverse Events (AEs)

No subject withdrew from the study due to an adverse event. All Snus products were well tolerated. One subject (No 10) experienced excessive salivation and irritated throat following administration of Snus E. The individual adverse events are tabulated in Tables 8-12.

Table 8. Adverse Events. Treatment A. 6 mg dose of Nicorette® Microtab sublingual nicotine tablets (=3 tablets).

Pat No	Adverse Event	Severity	Duration	Relationship*
	No AE			

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Table 9. Adverse Events. Treatment B. Swedish portion snus PSWM 0.5 g (16 mg nicotine/g).

Pat No	Adverse Event	Severity	Duration	Relationship*
	No AE			

Table 10. Adverse Events. Treatment C. Swedish portion snus PSWL 1.0 g (8 mg nicotine /g).

Pat No	Adverse Event	Severity	Duration	Relationship*
	No AE			

Table 11. Adverse Events. Treatment D. Swedish portion snus PSWL 1.0 g (16 mg nicotine /g)

Pat No	Adverse Event	Severity	Duration	Relationship*
	No AE			

Table 12. Adverse Events. Treatment E. Swedish portion snus PSWL (8 mg nicotine /g) 2x1.0 g

Pat No	Adverse Event	Severity	Duration	Relationship*
10	Salivation, irritated throat	1	10 min	Y

12.3 Deaths, Serious Adverse Events, and Other Significant Adverse Events (SAEs)

An adverse event that met one or more of the following criteria/outcomes was classified as serious:

- Death
- Life-threatening (i.e., immediate risk of death)
- In-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

12.3.1 Deaths

There were no deaths reported in this study.

12.3.2 Other Serious Adverse Events

There were no serious adverse events reported in this study.

12.4 Clinical Laboratory Evaluation

Clinical laboratory results are reported under Efficacy Evaluation. Please see Section 11. 2.

13 DISCUSSION AND OVERALL CONCLUSIONS

Niaura et al. (2005) recently demonstrated that a rapid-release nicotine gum reduced cue-provoked craving more rapidly compared to nicotine polacrilex chewing gum. Both moist snuff and Snus have shown fast delivery of nicotine to the bloodstream and fast onset of pharmacological effects that were also dose-dependent (Fant V et al. 1999, Lunell & Curvall 2011). Further, Thornley et al. (2009) demonstrated elevated nicotine plasma concentrations following dosing of Zonnic nicotine powder in pouch compared to Nicorette® Microtab 2 mg sublingual tablets. Therefore a closer look at the uptake of nicotine and the onset of subjective effects of single doses of Swedish Portion Snus was warranted.

We thus assessed the plasma nicotine levels achieved following administration of a single doses of four Swedish Snus products compared to 6 mg of Nicorette® Microtab. The resulting plasma concentration-time profiles showed close similarity between all four Swedish Snus products, but differed profoundly from that of 6 mg of Nicorette® Microtab. The Swedish Portion Snus containing 16 mg nicotine (Snus D) was very close to bioequivalent to 6 mg Nicorette sublingual nicotine tablets (Nicorette Microtab®) with respect to the nicotine uptake measured as AUC_{inf}. However, the two Snus products D and E, both containing 18 mg nicotine, were statistically bioequivalent. C_{max} of Snus D and Snus E, respectively, were 170% and 138% of the C_{max} of 6 mg of Nicorette® Microtab, demonstrating a comparatively

faster uptake. The difference in nicotine uptake rate between Swedish Portion Snus and Nicorette® Microtab in the present study may be explained by a longer than expected disintegration time of the tablets, 58.4 ±12.3 minutes, compared to a published study, about 30 minutes (Molander L and Lunell E 2001). Formation of a larger aggregate in the oral cavity of the subjects may be one reason. Subjects were instructed to keep the tablets still and not to suck on them or chew them.
As expected the mean plasma nicotine concentrations following the two 8 mg products Snus B and C, were lower compared to both 16 mg products, Snus D and E. See Figure 1.

All products including the sublingual tablet generated elevation of the subjective effect "head rush" over the first 30 minutes when recordings were made (Figure 3). "Head rush" mirrors a pharmacological effect in the "reward" system of the brain and is of paramount importance for the subject's liking of a nicotine containing product. Only minor differences in craving were seen over the first 30 minutes. Craving constitutes the main nicotine "withdrawal" symptom.

The 6 mg dose of Nicorette® Microtab produced a somewhat larger AUC_{inf} than both the higher nicotine doses of snus. The AUC_{inf} of the Snus D, PSWL 1g (16 mg nicotine /g), thus was 93.8% and that of Snus E, PSWL 2x1 g (8mg nicotine /g), was 88.7% of the AUC_{inf} after administration of 6 mg of Nicorette® Microtab.

Mean C_{max} of the various Swedish Snus products, except for Snus C, was higher than for the 6 mg dose of Nicorette® Microtab. More importantly, the median T_{max} was shorter, 24-30 minutes, for all four strengths of Swedish Snus compared to 105 minutes for the 6 mg of Nicorette® Microtab. The lower C_{max} of the 6 mg of Nicorette® Microtab compared to three out of the four Swedish Snus, in spite of a larger AUC_{inf} may be explained by a slower and more prolonged absorption from the 6 mg of Nicorette® Microtab.
As demonstrated in Figure 2, the relationship between In-vivo extracted dose/kg Bw and nicotine uptake measured as Area Under the plasma Concentration – Time curve (AUC_{inf}) showed fairly good linearity, particularly when the extracted dose was corrected for body surface area (BSA).
One interesting observation is that the mean extracted amount of nicotine from Snus B, portion size 0.5 g, was 22% larger, AUC_{inf} was 35% larger , and C_{max} was 34% larger, compared to Snus C, portion size 1 g, both portions containing 8 mg nicotine. It could thus be concluded

that extraction as well as uptake and rate of uptake seemed to be better from the smaller pouch of snus. This is in agreement with a previous multiple-dose study (Lunell E & Lunell M 2005). That study showed that the AUC and C_{max} values of Swedish Snus PSWL,Catch Licorice, 1 g portion snus containing 8 mg nicotine, did not differ significantly from that of Swedish Snus PSOM, Catch Mini 0.5 g portion snus, containing 4 mg nicotine, despite the twice-as-large amount of snus. The higher bioavailability found for the smaller pouch may be related to better penetration of saliva as well as shorter distance for diffusion of nicotine out from the pouch, leading to more efficient absorption of the extracted dose.

This study assessed nicotine extraction and systemic uptake from pouched SS products. The similar nicotine absorption for 1 g SS portions of 16mg and two 1 g SS portions, 2 x 8mg, indicates that absorption kinetics were dependent on total nicotine extraction rather than mode of administration.

The inter-individual extraction showed quite high variability, from 10 to 57%. However, the intra-individual variability between the four different snus products (B, C, D, E) was small. Each subject showed a high consistency with respect to extraction. Individual factors such as salivation and discreet oral movements may separate the two groups, however were not possible to measure.

The relationship between the AUC_{inf} and the *In-vivo* extracted dose per kg body weight showed fairly good linearity. The extracted amount of nicotine from different preparations of pouched SS thus provides a good prediction of the systemic exposure to nicotine, when humidity and pH are identical.

The SS portions of a total of 16mg showed essential similarity, pharmacokinetic (AUC) as well as pharmacodynamic (VAS scores), to the 6 mg of Nicorette® Microtab sublingual tablets. According to the results of the present study, however, the Swedish Portion Snus products, 8mg as well as 16mg strengths, produced higher maximum blood nicotine concentrations in shorter time, compared to the Microtab.

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14 TABLES AND FIGURES REFERRED TO BUT NOT INCLUDED IN THE TEXT

(b) (6)

14.1 Individual Patient Data Listings

Pat No	Init	Sex	Age (yrs)	Height (cm)	Weight (kg)	Portions /day	Since (yrs)	Brand	Ex-smoker
1	(b)	M	19	174	72	13	4	Swedsnus	No
2	(b)	M	43	183	90	24	29	Granit	Yes
3	(b)	M	20	173	80	12	3	General	Yes
4	(b)	M	46	189	99	18	25	General	Yes
5	(b)	M	49	176	88	42	15	General	Yes
6	(b)	M	33	185	110	10	9	Gbg Rapé	Yes
7	(b)	M	39	185	129	30	25	Granit	No
8	(b)	M	21	184	90	10	6	General	No
9	(b)	M	23	170	57	12	6	Gbg Rapé	No
10	(b)	M	19	185	81	6	2	General	Yes
11	(b)	M	58	184	99	30	25	Kaliber	No
12	(b)	F	50	164	56	10	10	Catch liq	Yes
13	(b)	M	52	178	86	7	20	Probe	Yes
14	(b)	M	47	180	90	11	30	General	Yes
15	(b)	F	45	181	70	11	10	Catch White	Yes
16	(b)	M	19	170	54	18	5	Kaliber	No
17	(b)	M	20	190	80	10	2	General	No
18	(b)	M	19	185	92	14	5	General	No

15 INDIVIDUAL NICOTINE PLASMA CONCENTRATION-TIME CURVES AND PHARMACOKINETIC PARAMETERS

15.1 Nicotine extraction from Snus B – E

Table 15.1.a. Nicotine extraction from Snus B, PSWM 0.5 g (16 mg nicotine/g)

Subject No	Residual amount (mg)	Extraction* (mg)	Extraction (%)
1	4.66	2.65	36
2	5.98	1.33	18
3	6.54	0.77	11
4	4.77	2.54	35
5	6.12	1.19	16
6	6.10	1.21	17
7	5.57	1.74	24
8	4.92	2.39	33
9	6.33	0.98	13
10	5.18	2.13	29
11	5.62	1.69	23
12	3.46	3.85	53
13	4.07	3.24	44
14	6.00	1.31	18
15	5.19	2.12	29
16	5.53	1.78	24
17	6.19	1.12	15
18	5.21	2.10	29
Mean	5.41	1.90	26
SD	0.82	0.82	11.21
Min	3.46	0.77	11
Max	6.54	3.85	53

*Mean of 10 unused portions of Snus B (7.31) minus residual amount

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Table 15.1.b. Nicotine extraction from Snus C, PSWL 1.0 g (8 mg nicotine /g)

Subject No	Residual amount (mg)	Extraction* (mg)	Extraction (%)
1	6.06	1.66	22
2	6.58	1.14	15
3	6.60	1.12	15
4	5.15	2.57	33
5	6.92	0.80	10
6	6.64	1.08	14
7	6.87	0.85	11
8	6.27	1.45	19
9	6.83	0.89	12
10	6.42	1.30	17
11	6.82	0.90	12
12	3.31	4.41	57
13	5.11	2.61	34
14	6.80	0.92	12
15	4.93	2.79	36
16	6.65	1.07	14
17	6.50	1.22	16
18	6.43	1.29	17
Mean	6.16	1.56	20
SD	0.95	0.95	12.27
Min	3.31	0.80	10
Max	6.92	4.41	57

*Mean of 10 unused portions of Snus C (7.72) minus residual amount

Date: 2013-06-17

Table 15.1.c. Nicotine extraction from Snus D, PSWL 1.0 g (16 mg nicotine /g)

Subject No	Residual amount (mg)	Extraction* (mg)	Extraction (%)
1	12.1	3.6	23
2	13.4	2.3	15
3	14.1	1.6	10
4	11.5	4.2	27
5	13.8	1.9	12
6	13.8	1.9	12
7	13.2	2.5	16
8	12.9	2.8	18
9	14.1	1.6	10
10	12.6	3.1	20
11	13.6	2.1	13
12	6.95	8.8	56
13	11.4	4.3	27
14	13.7	2.0	13
15	12.0	3.7	24
16	12.9	2.8	18
17	13.1	2.6	17
18	13.3	2.4	15
Mean	12.7	3.0	19
SD	1.65	1.65	10.54
Min	14.1	8.8	56
Max	7.0	1.6	10

*Mean of 10 unused portions of Snus D (15.7 mg) minus residual amount

Date: 2013-06-17
Table 15.1.d. Nicotine extraction from E = PSWL (8 mg nicotine /g) 2x1.0 g

Subject No	Residual amount (mg)	Extraction* (mg)	Extraction (%)
1	11.2	4.2	27
2	12.7	2.7	18
3	13.5	1.9	12
4	11.7	3.7	24
5	13.8	1.6	10
6	13.2	2.2	14
7	13.0	2.4	16
8	12.8	2.6	17
9	13.1	2.3	15
10	12.2	3.2	21
11	13.2	2.2	14
12	9.52	5.9	38
13	10.7	4.7	31
14	13.8	1.6	10
15	9.41	6.0	39
16	12.6	2.8	18
17	13.5	1.9	12
18	13.0	2.4	16
Mean	12.4	3.0	20
SD	1.35	1.35	8.80
Min	9.41	1.6	10
Max	13.8	6.0	39

*Mean of 10 unused portions of 2xSnus C (15.4 mg) minus residual amount

Date: 2013-06-17
Table 15.1.e. Nicotine extraction

Subject No	Snus B Extraction (%)	Snus C Extraction (%)	Snus D Extraction (%)	Snus E Extraction (%)	Mean Individ	Min Individ	Max Individ	High/ Low
1	36	22	23	27	27	22	36	H
2	18	15	15	18	16,5	15	18	L
3	11	15	10	12	12	10	15	L
4	35	33	27	24	29,75	24	35	H
5	16	10	12	10	12	10	16	L
6	17	14	12	14	14,25	12	17	L
7	24	11	16	16	16,75	11	24	L
8	33	19	18	17	21,75	17	33	H
9	13	12	10	15	12,5	10	15	L
10	29	17	20	21	21,75	17	29	H
11	23	12	13	14	15,5	12	23	L
12	53	57	56	38	51	38	57	H
13	44	34	27	31	34	27	44	H
14	18	12	13	10	13,25	10	18	L
15	29	36	24	39	32	24	39	H
16	24	14	18	18	18,5	14	24	H
17	15	16	17	12	15	12	17	L
18	29	17	15	16	19,25	15	29	H
Mean Group	26	20	19	20	Median 17,625			
SD	11.21	12.27	10.54	8.80				
Min	11	10	10	10				
Max	53	57	56	39				

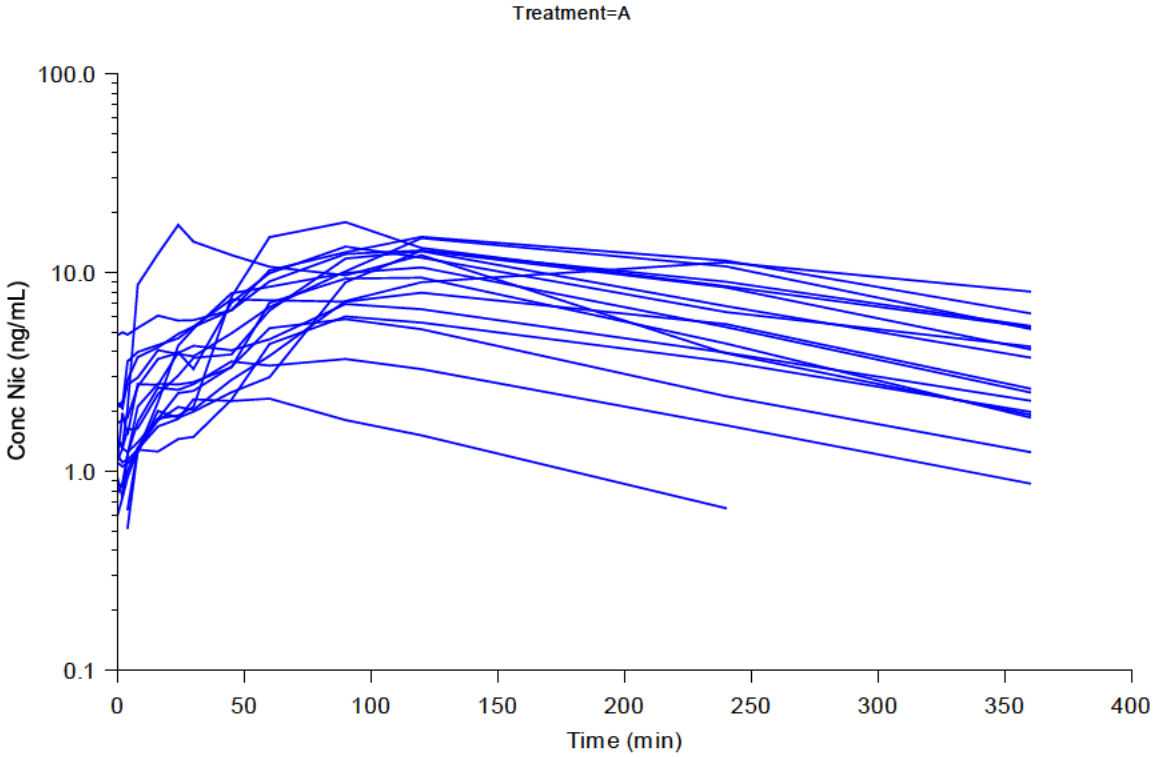
Date: 2013-06-17

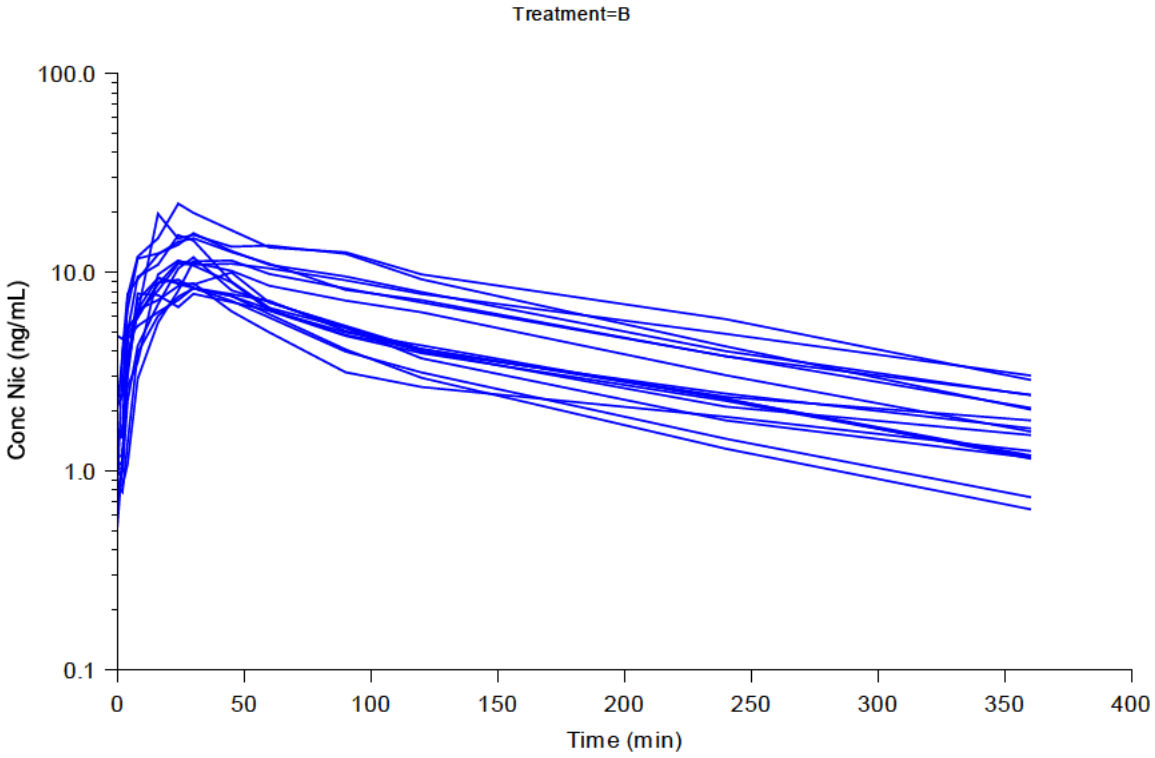
Table 15.1.f. *In-vivo* Disintegration time of 6 mg dose of sublingual nicotine tablets (=3 tablets).

Subject No	Disintegration time (minutes)	Comments
1	80	
2*	56	
3	30	
4	64	
5	60	
6	70	
7	62	
8	59	
9	60	
10	45	
11	66	
12	30	
13	60	
14	60	
15	60	
16	63	
17*	61	
18	65	
Mean	58.39	
SD	12.33	
Min	30	
Max	80	

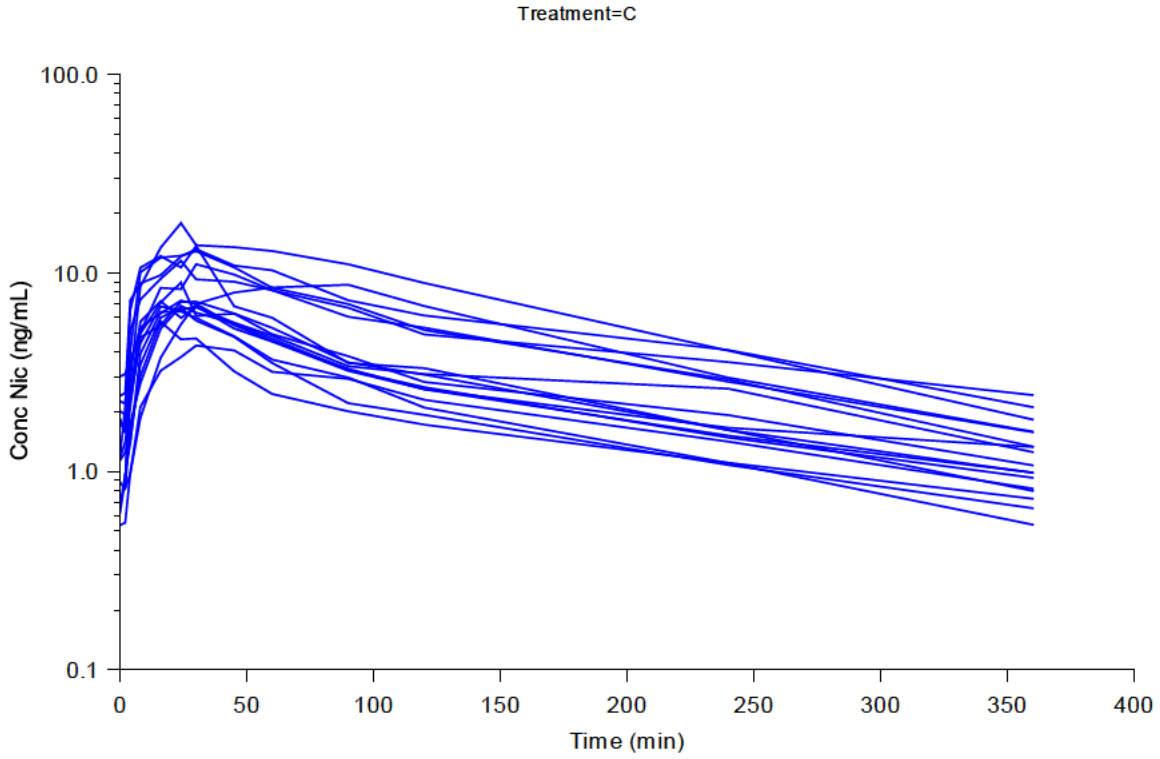
Date: 2013-06-17

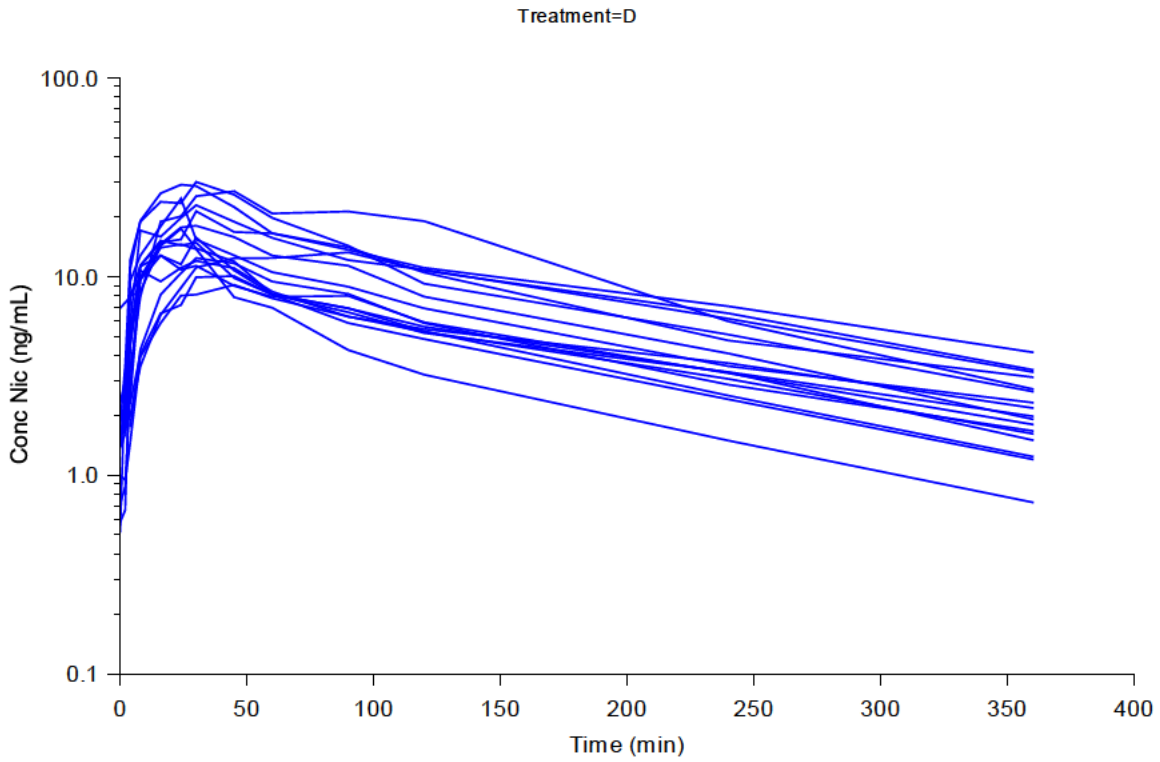
15.2 Spaghetti plots by Treatment



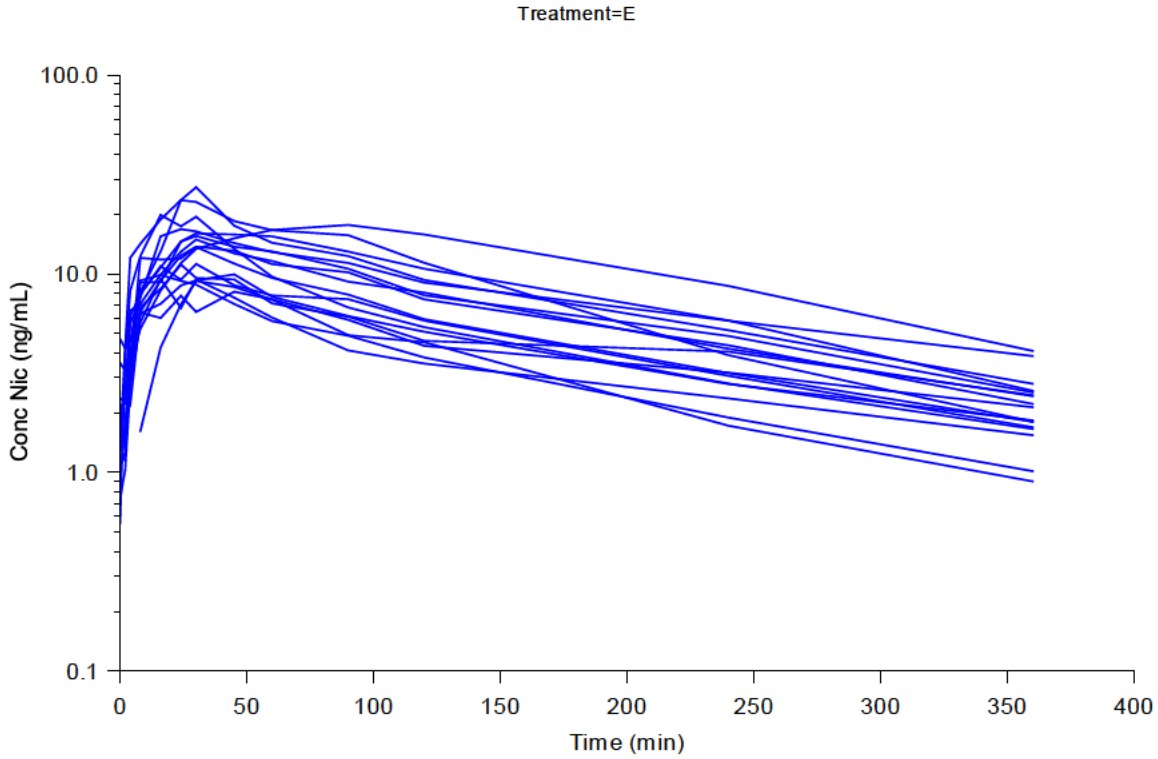


Date: 2013-06-17





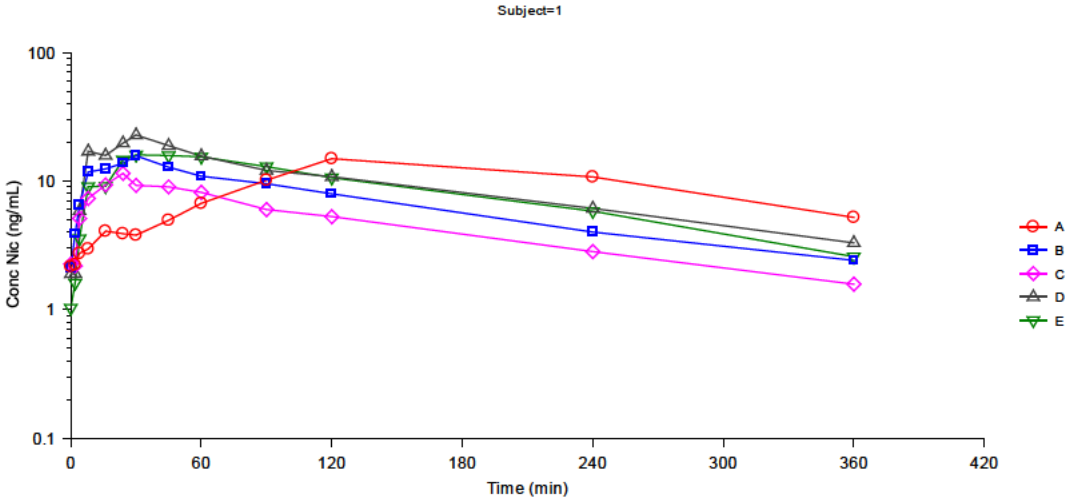
Date: 2013-06-17

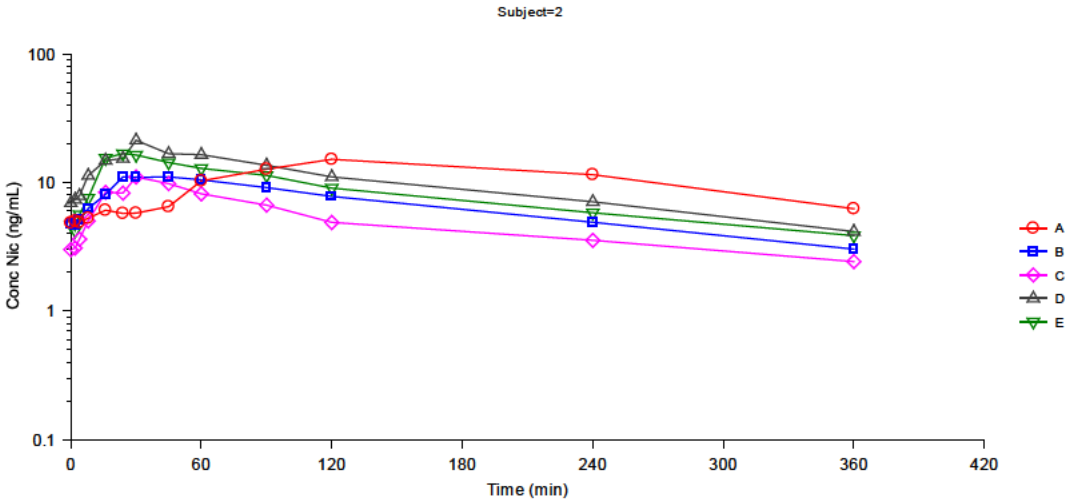


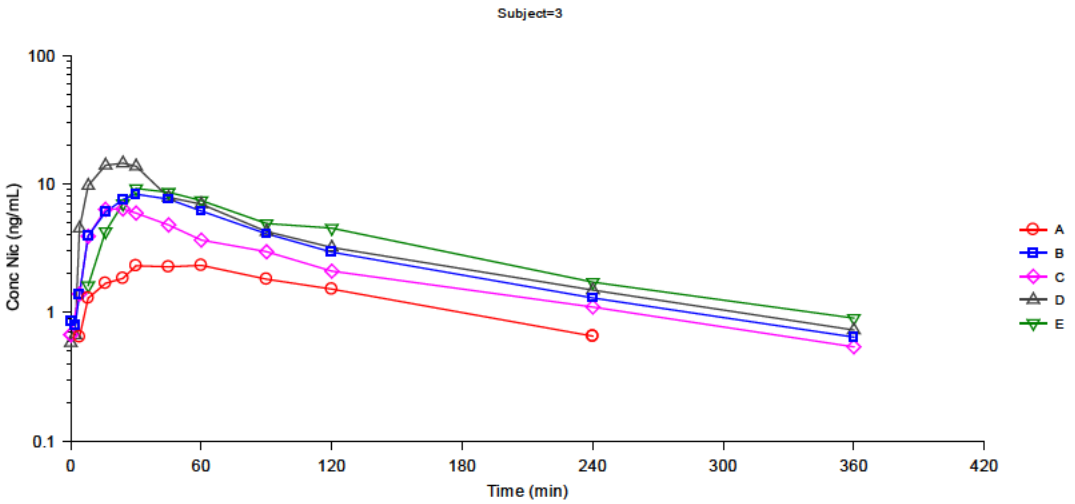
Date: 2013-06-17

15.3 Individual Nicotine plasma concentration-time curves.

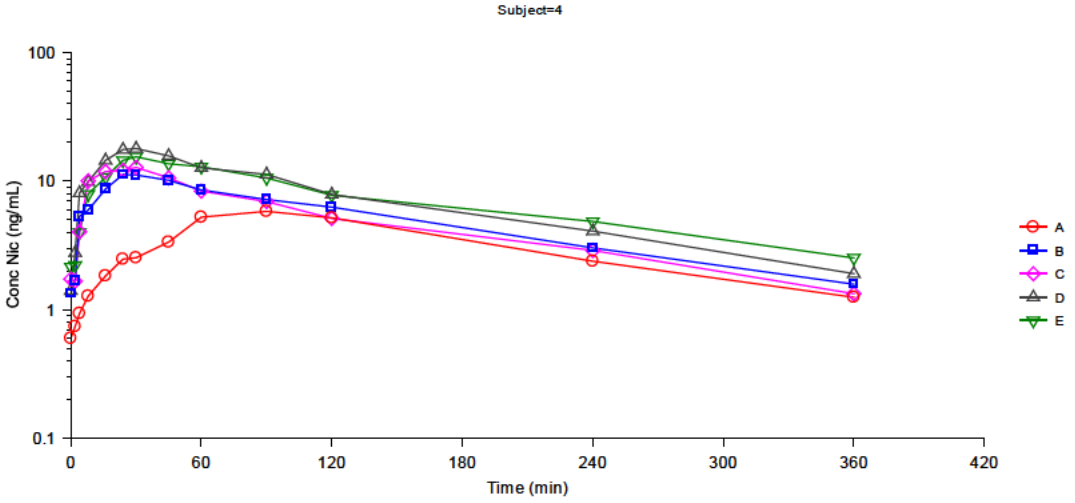
Spaghetti plots by subject.

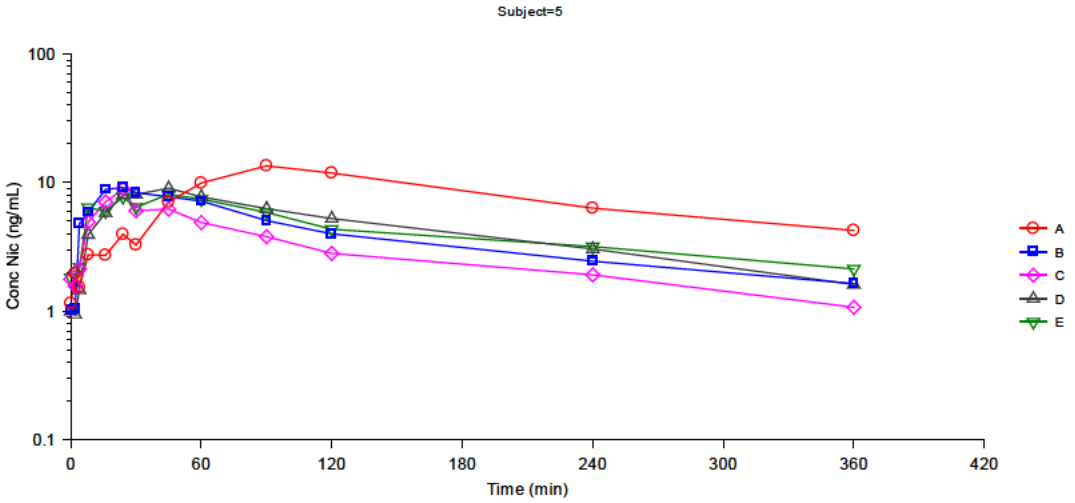


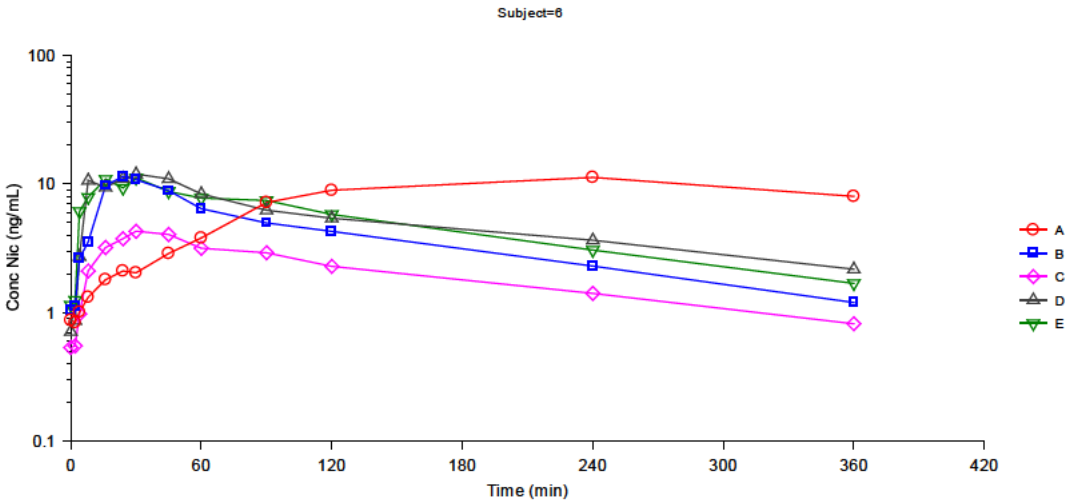




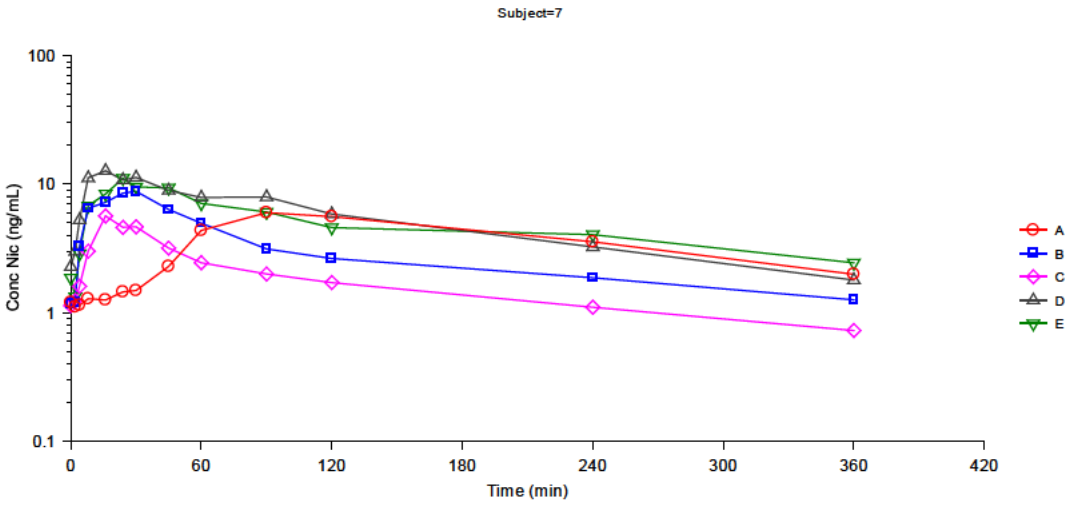
Date: 2013-06-17

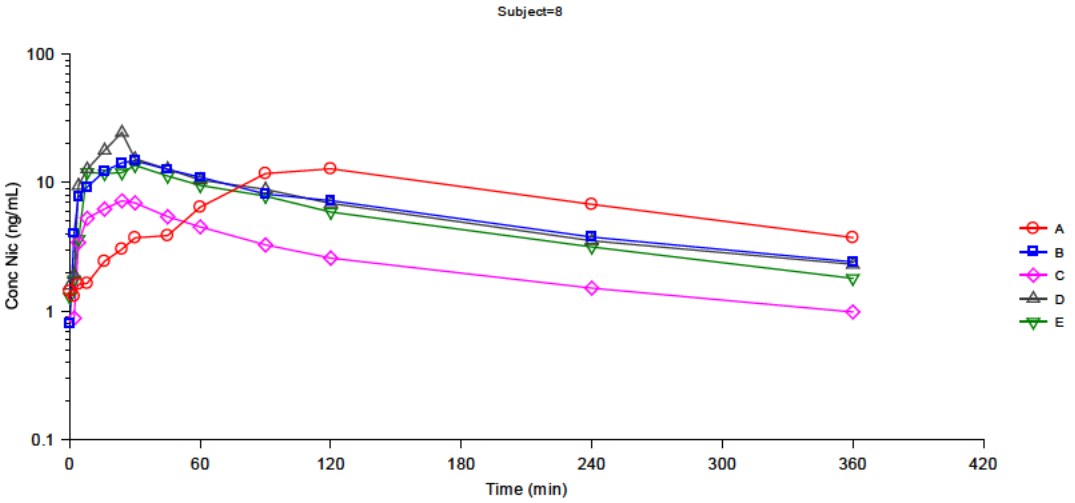


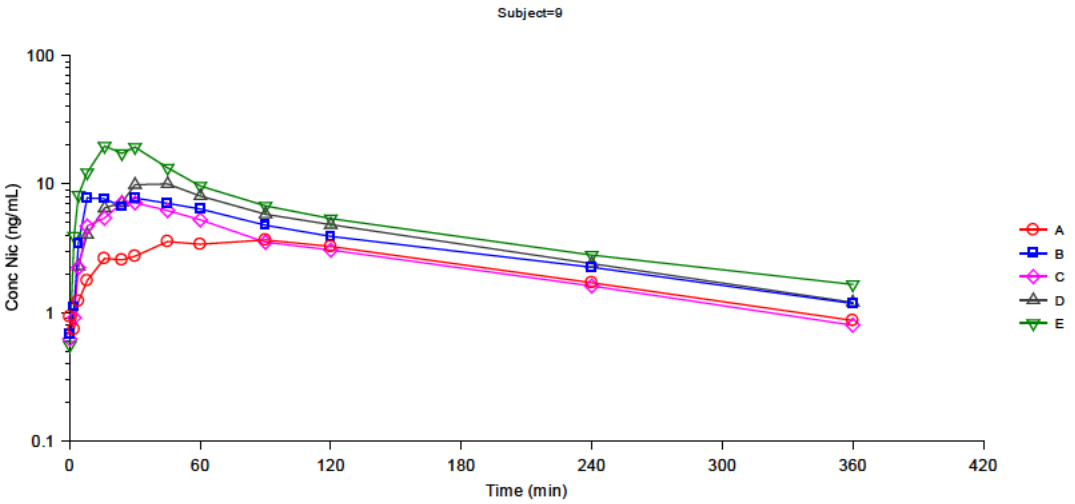




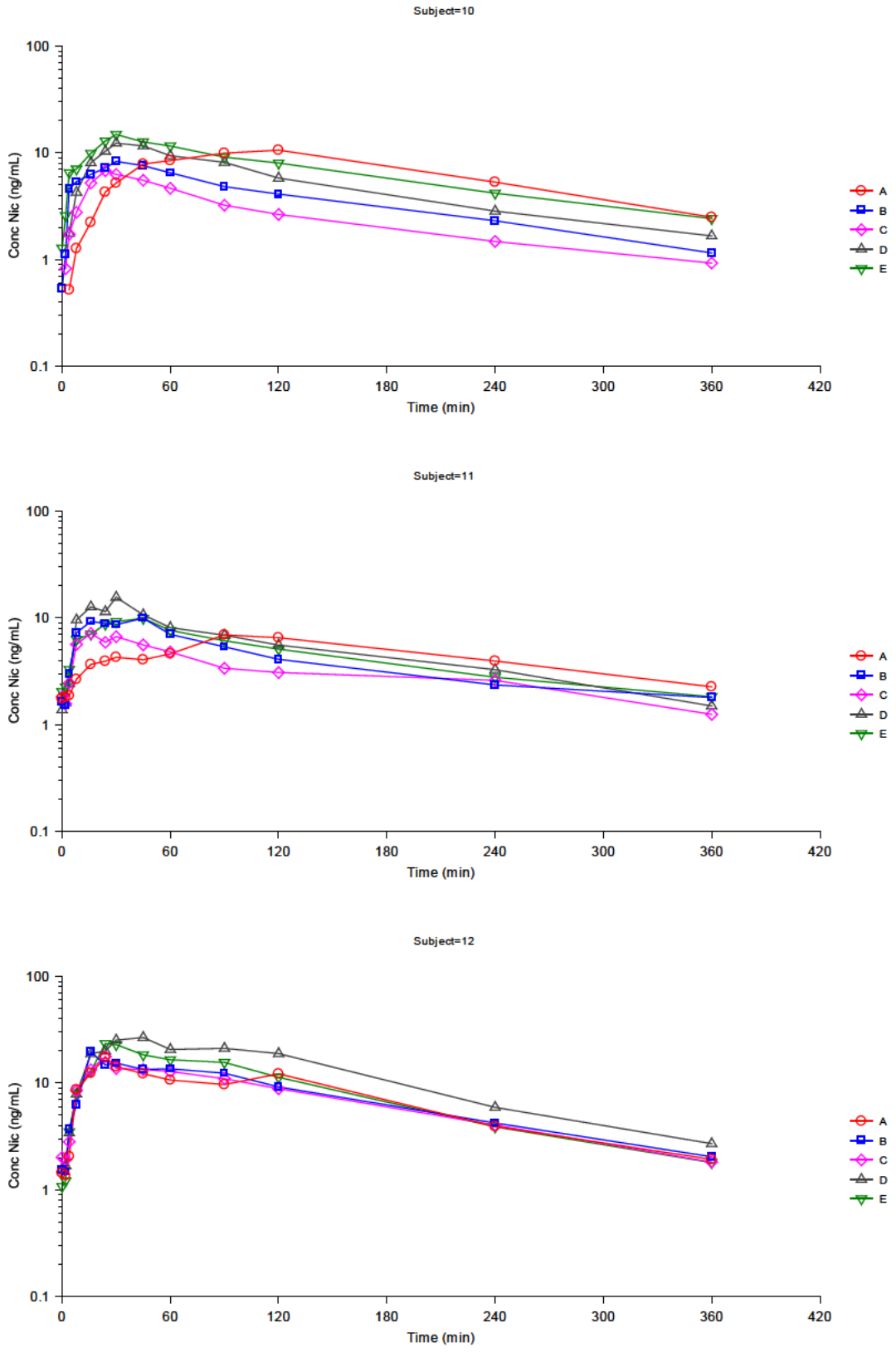
Date: 2013-06-17



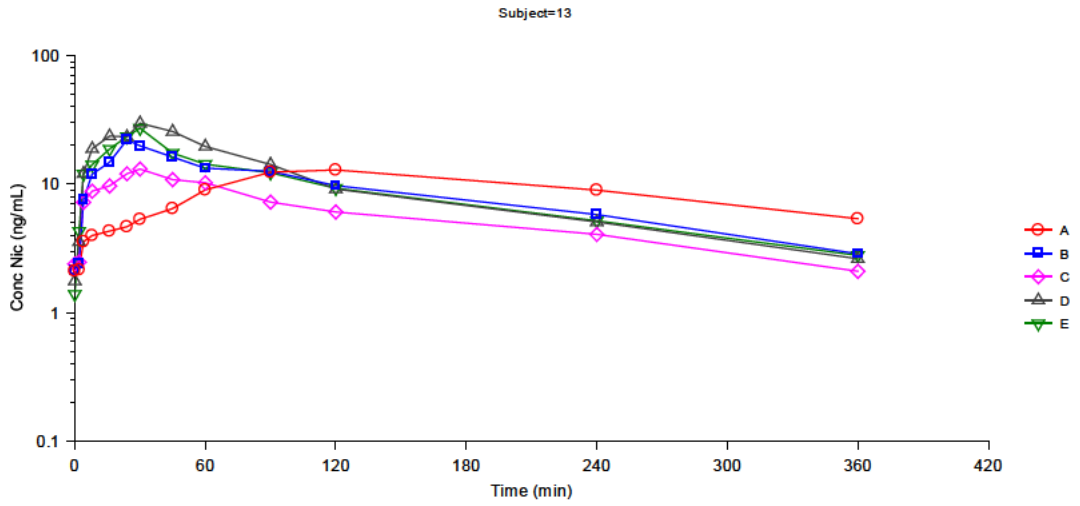


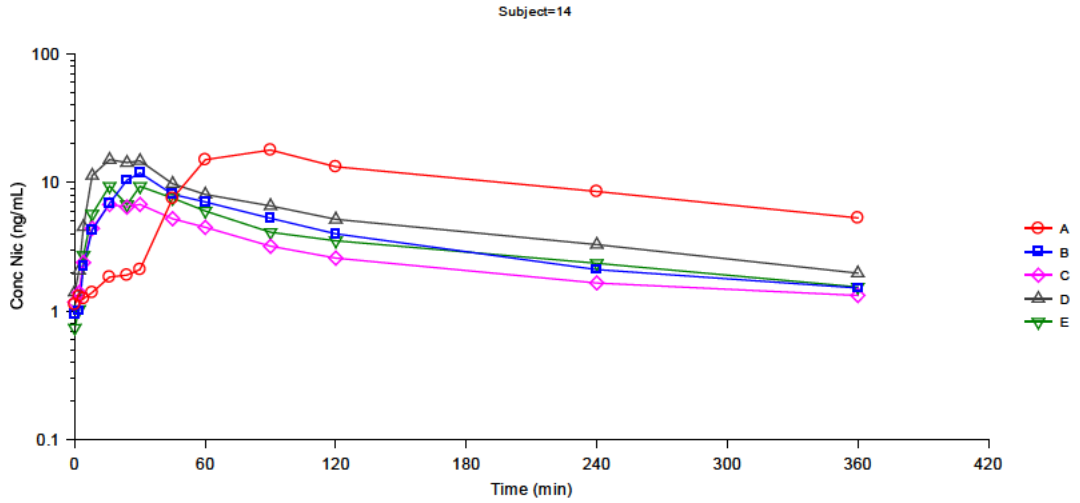


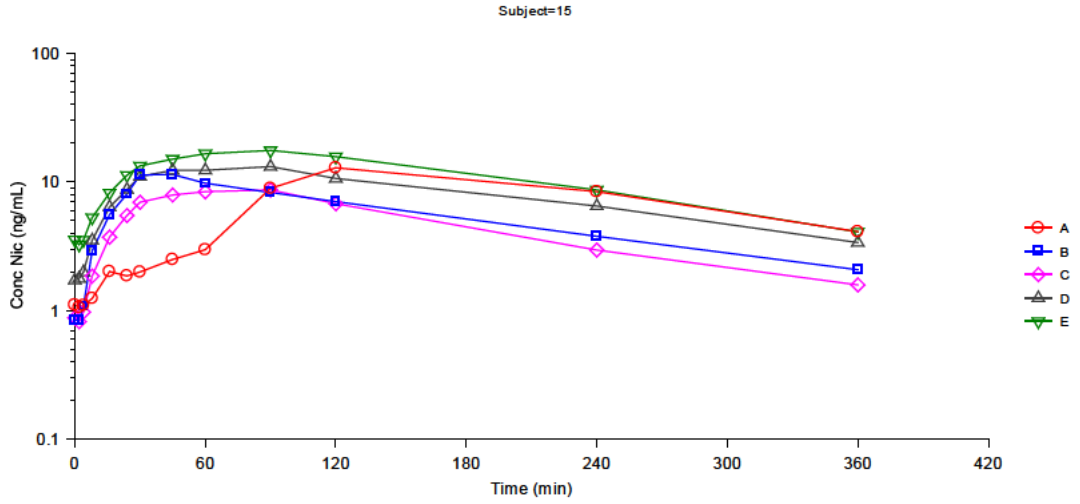
Date: 2013-06-17



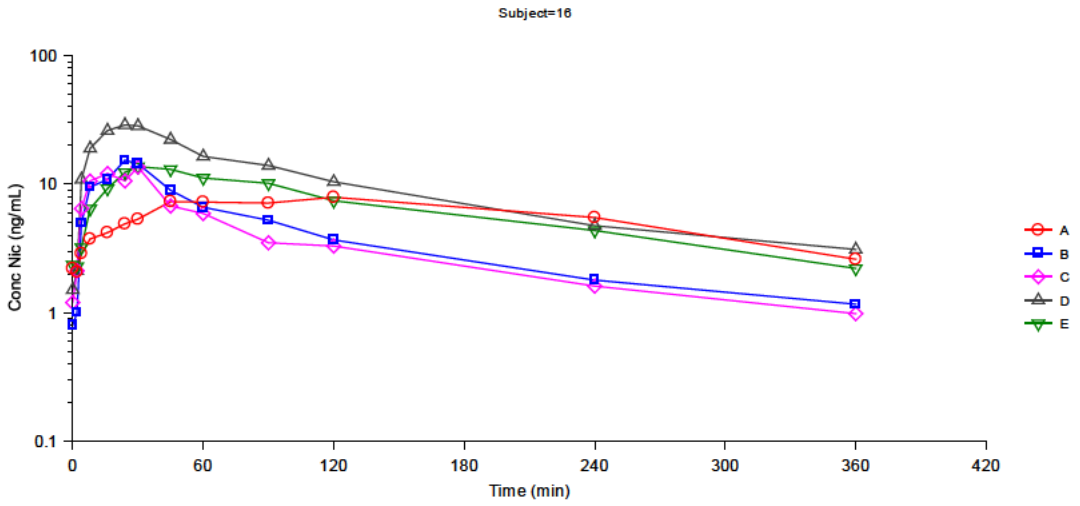
Date: 2013-06-17

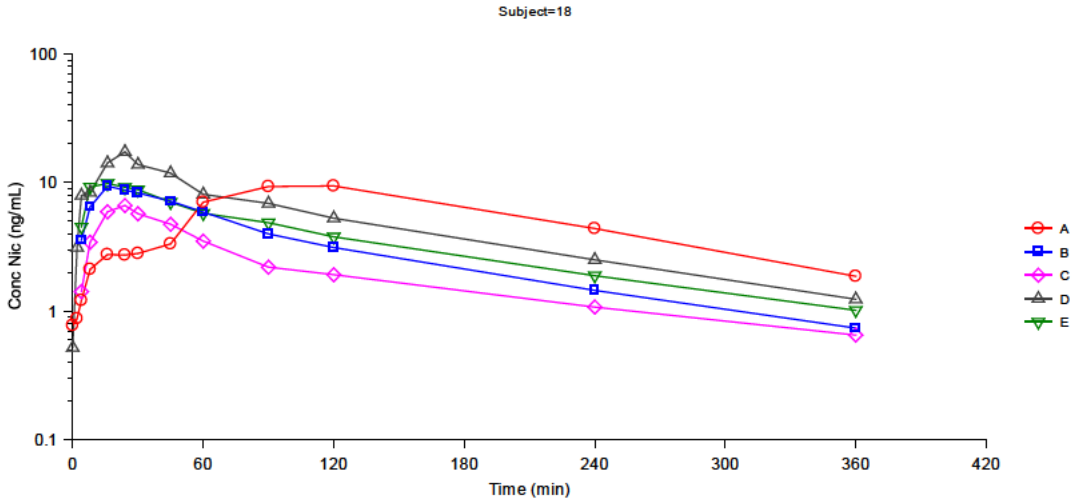






Date: 2013-06-17





Date: 2013-06-17

15.4 INDIVIDUAL NICOTINE PLASMA VALUES ng/mL

Treatment A. 6 mg dose of Nicorette sublingual nicotine tablets (=3 tablets). N=16.

Subj /time	1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	Mean	SD
0	2,16	0	0,60	1,15	0,87	1,20	1,42	0,93	0	1,75	1,44	2,12	1,13	1,11	2,21	0,774	1,11	0,64
2	2,23	0,50	0,74	1,96	0,84	1,11	1,31	0,74	0,50	1,80	1,36	2,16	1,31	1,06	2,08	0,875	1,33	0,51
4	2,73	0,64	0,93	1,53	1,01	1,15	1,62	1,23	0,52	1,87	2,06	3,57	1,25	1,10	2,89	1,21	1,51	0,82
8	2,96	1,29	1,28	2,75	1,32	1,29	1,65	1,77	1,27	2,67	8,68	3,99	1,40	1,24	3,75	2,12	2,43	1,96
16	4,07	1,68	1,84	2,72	1,80	1,26	2,45	2,64	2,23	3,68	12,4	4,32	1,84	2,01	4,20	2,76	3,19	2,71
24	3,89	1,84	2,48	3,97	2,11	1,45	3,04	2,57	4,29	3,94	17,3	4,67	1,91	1,86	4,92	2,74	3,94	3,86
30	3,79	2,30	2,54	3,27	2,04	1,49	3,72	2,75	5,22	4,28	14,3	5,33	2,11	2,00	5,35	2,81	3,97	3,13
45	4,95	2,26	3,36	7,06	2,89	2,29	3,87	3,57	7,86	4,07	12,3	6,47	7,55	2,50	7,33	3,34	5,11	2,86
60	6,70	2,32	5,25	9,93	3,81	4,37	6,45	3,40	8,48	4,61	10,7	9,03	15,1	2,98	7,26	7,04	6,71	3,37
90	10,1	1,81	5,83	13,5	7,19	6,01	11,8	3,68	9,95	6,95	9,69	12,4	17,9	8,94	7,14	9,33	8,8	4,05
120	14,9	1,52	5,19	11,9	8,95	5,60	12,8	3,27	10,6	6,54	12,2	12,9	13,3	12,9	7,92	9,42	9,00	3,84
240	10,8	0,65	2,39	6,33	11,3	3,57	6,78	1,71	5,32	3,95	3,93	8,99	8,54	8,43	5,51	4,40	5,45	2,97
360	5,21	0,50	1,25	4,25	8,03	1,99	3,74	0,87	2,50	2,27	1,93	5,39	5,32	4,12	2,61	1,87	3,30	1,97

Date: 2013-06-17
Treatment B. PSWM 0,5 g (16 mg nicotine/g). N=16.

Subject /time	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	M	SD
0	2,10	2,10	0,85	1,34	1,02	1,04	1,17	0,80	0,67	0,53	1,65	1,53	2,13	0,95	0,84	0,80	0	1,09	0,56
2	3,88	3,88	0,79	1,68	1,04	1,13	1,20	4,03	1,10	1,13	1,51	1,47	2,40	1,01	0,84	1,01	0.50	1,61	1,03
4	6,51	6,51	1,38	5,34	4,80	2,65	3,28	7,75	3,45	4,62	2,98	3,71	7,58	2,24	1,08	4,99	3,57	4,12	1,99
8	11,7	11,7	3,94	5,96	5,92	3,53	6,48	9,23	7,81	5,37	7,26	6,23	12,0	4,26	2,91	9,50	6,47	6,79	2,71
16	12,4	12,4	6,02	8,70	8,85	9,73	7,24	12,3	7,67	6,29	9,31	19,7	14,8	6,90	5,58	10,9	9,39	9,74	3,68
24	13,8	13,8	7,54	11,3	9,17	11,5	8,52	14,2	6,68	7,24	8,87	14,8	22,1	10,5	8,08	15,4	8,74	11,15	4,06
30	15,7	15,7	8,30	11,2	8,34	10,8	8,80	14,8	7,78	8,33	8,67	15,4	19,9	11,9	11,4	14,4	8,32	11,50	3,59
45	12,8	12,8	7,60	10,2	7,76	8,90	6,36	12,6	7,08	7,59	9,97	13,4	16,3	8,11	11,5	8,97	7,18	9,77	2,82
60	10,9	10,9	6,14	8,55	7,18	6,43	4,98	11,0	6,40	6,51	7,03	13,6	13,3	7,08	9,80	6,62	5,91	8,21	2,69
90	9,52	9,52	4,09	7,20	5,07	4,98	3,14	8,15	4,78	4,83	5,37	12,4	12,6	5,29	8,29	5,25	3,99	6,56	2,89
120	7,95	7,95	2,94	6,29	4,00	4,28	2,65	7,24	3,92	4,11	4,10	9,21	9,75	4,00	7,05	3,69	3,13	5,27	2,29
240	4,01	4,01	1,29	3,03	2,45	2,30	1,87	3,77	2,25	2,31	2,36	4,23	5,81	2,11	3,78	1,80	1,45	2,80	1,21
360	2,41	2,41	0,64	1,58	1,64	1,20	1,26	2,42	1,18	1,16	1,80	2,04	2,88	1,52	2,08	1,17	0,74	1,61	0,63

Date: 2013-06-17
Treatment C. PSWL 1,0 g (8 mg nicotine /g). N=17.

Subject /time	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	M	SD
0	2,26	3,00	0,67	1,74	1,77	0,54	1,14	0.50	0,62	0.50	1,83	2,01	2,40	1,17	0,88	1,20	0,00	1,42	0,82
2	2,19	3,09	0 50	1,67	1,57	0,55	1,24	0,88	0,91	0,82	1,57	1,92	2,48	1,39	0,82	2,13	0.50	1,55	0,72
4	5,11	3,62	1,39	4,04	2,13	0,98	1,61	3,42	2,24	1,73	2,46	2,82	7,24	2,39	0,97	6,48	1,42	2,94	1,85
8	7,32	5,01	3,90	10,00	4,93	2,11	3,02	5,29	4,72	2,78	5,67	8,51	8,83	4,41	1,86	10,6	3,44	5,44	2,71
16	9,32	8,38	6,34	11,9	7,07	3,21	5,66	6,24	5,44	5,22	7,17	13,4	9,71	6,76	3,73	12,1	5,93	7,50	2,91
24	11,5	8,26	6,38	12,2	8,92	3,76	4,62	7,23	7,12	6,79	5,92	17,7	12,1	6,47	5,49	10,6	6,64	8,33	3,50
30	9,25	11,0	5,92	12,8	6,05	4,30	4,66	6,96	7,15	6,29	6,69	13,7	13,1	6,77	6,96	13,6	5,73	8,30	1,15
45	9,00	9,78	4,78	10,6	6,22	4,06	3,19	5,44	6,23	5,54	5,60	13,4	10,9	5,23	7,93	6,77	4,75	7,02	2,80
60	8,15	8,15	3,65	8,38	4,90	3,17	2,45	4,53	5,27	4,66	4,81	12,8	10,3	4,50	8,41	5,92	3,51	6,09	2,82
90	6,00	6,65	2,97	6,94	3,80	2,92	2,00	3,28	3,53	3,23	3,38	11,0	7,26	3,21	8,69	3,51	2,20	4,74	2,55
120	5,28	4,89	2,09	5,14	2,82	2,29	1,72	2,59	3,07	2,65	3,09	8,87	6,08	2,59	6,79	3,30	1,92	3,83	2,01
240	2,82	3,55	1,10	2,90	1,92	1,41	1,10	1,51	1,61	1,48	2,61	4,05	4,08	1,66	2,96	1,61	1,08	2,20	1,02
360	1,57	2,42	0,54	1,33	1,07	0,82	0,73	0,990	0,798	0,93	1,25	1,82	2,10	1,33	1,58	0,98	0,652	1,23	0,53

Date: 2013-06-17
Treatment D. PSWL 1,0 g (16 mg nicotine /g). N=16

Subject /time	1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	M	SD
0	1,89	0,58	1,41	1,00	0,71	2,29	1,58	0,00	0,00	1,38	1,57	1,77	1,41	1,73	1,51	0,52	1,21	0,67
2	1,90	0,67	2,78	0,94	0,87	2,99	1,93	0.50	0.50	1,61	1,68	3,57	2,08	1,78	2,12	3,14	2,00	0,87
4	5,85	4,50	8,10	1,46	2,73	5,26	9,48	2,30	1,76	2,44	3,42	12,1	4,54	1,99	10,9	7,95	5,30	3,43
8	17,0	9,68	9,90	3,94	10,7	11,2	12,7	4,04	4,28	9,60	7,91	18,9	11,4	3,52	19,0	8,38	10,13	4,99
16	15,8	13,9	14,5	5,81	9,42	12,7	17,8	6,50	8,09	12,7	18,8	23,7	15,1	6,40	26,1	14,2	13,86	5,91
24	19,7	14,5	17,6	8,00	11,0	10,8	24,5	7,17	10,4	11,5	20,0	23,3	14,3	8,75	28,9	17,3	15,49	6,46
30	22,8	13,7	18,0	8,09	11,9	11,3	15,3	9,88	12,3	15,7	25,3	29,8	14,8	11,1	28,4	13,8	16,38	6,67
45	18,8	7,85	15,7	9,05	11,0	8,95	12,7	10,0	11,6	10,7	26,8	25,7	9,80	12,3	22,3	11,9	14,08	6,06
60	15,6	6,95	12,7	7,75	8,38	7,89	10,5	8,07	9,41	8,13	20,7	19,7	8,09	12,4	16,5	8,11	11,30	4,50
90	12,1	4,26	11,3	6,27	6,26	7,96	8,86	5,82	8,15	6,90	21,2	14,2	6,59	13,2	14,0	6,90	9,62	4,40
120	10,8	3,20	7,89	5,26	5,42	5,86	6,89	4,83	5,81	5,58	18,9	9,18	5,19	10,7	10,4	5,29	7,58	3,81
240	6,12	1,49	4,09	3,05	3,66	3,25	3,53	2,41	2,85	3,28	5,93	5,09	3,30	6,51	4,75	2,51	3,86	1,44
360	3,30	0,73	1,91	1,61	2,18	1,80	2,32	1,20	1,67	1,50	2,71	2,63	1,98	3,39	3,11	1,24	2,08	0,78

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Treatment E. PSWL (8 mg nicotine /g) 2x1,0 g. N=16

Subject /time	1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	M	SD
0	1,02	0.00	2,16	1,82	1,14	1,87	1,29	0,56	1,28	2,04	1,07	1,39	0,74	3,56	2,35	0.00	1,59	0,78
2	1,61	0.25	2,22	1,90	1,25	1,33	1,75	3,94	2,58	2,26	1,21	4,31	1,03	3,26	2,29	0.50	2,21	0,98
4	3,56	0.50	4,00	2,20	6,13	2,88	3,62	8,21	6,53	3,30	3,50	12,0	2,72	3,54	3,21	4,53	4,66	2,51
8	9,05	1,62	7,93	6,38	7,89	6,80	12,0	12,3	7,06	6,31	8,13	14,2	5,70	5,29	6,44	9,24	7,89	3,04
16	9,10	4,26	10,8	5,99	10,9	8,40	11,8	19,7	9,90	7,06	13,0	18,8	9,39	8,24	9,26	9,86	10,40	4,06
24	14,5	7,02	14,5	7,75	9,33	11,1	11,9	17,3	12,9	8,73	23,5	23,5	6,73	11,3	12,4	9,21	12,61	5,14
30	16,0	9,19	15,5	6,43	11,2	9,52	13,6	19,4	14,9	9,26	23,0	27,3	9,36	13,4	13,7	8,81	13,77	5,61
45	15,8	8,59	13,7	8,12	8,76	9,36	11,3	13,4	12,7	9,93	18,4	17,5	7,58	15,1	13,1	7,05	11,88	3,59
60	15,4	7,41	13,0	7,44	7,77	7,09	9,53	9,65	11,6	7,65	16,6	14,3	6,02	16,6	11,2	5,77	10,43	3,75
90	12,9	4,91	10,6	5,86	7,45	6,08	7,85	6,77	9,13	6,12	15,6	12,3	4,11	17,6	10,2	4,88	8,90	4,01
120	10,6	4,52	7,78	4,34	5,80	4,60	5,91	5,39	8,04	5,11	11,4	9,30	3,53	15,8	7,43	3,79	7,08	3,22
240	5,82	1,72	4,86	3,18	3,07	4,05	3,16	2,80	4,19	2,79	3,89	5,20	2,36	8,68	4,37	1,89	3,88	1,73
360	2,58	0,902	2,53	2,13	1,69	2,45	1,80	1,66	2,42	1,83	1,80	2,80	1,54	4,09	2,21	1,02	2,09	0,76

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15.5 INDIVIDUAL PHARMACOKINETIC PARAMETERS.

C_{max} (µg/ml). N=16

Subj	Treat	C_{max}	Treat	C_{max}	Treat	C_{max}	Treat	C_{max}	Treat	C_{max}
1	A	14,9060	B	15,7060	C	11,4690	D	22,7840	E	15,9670
2*	A		B		C		D		E	
3	A	2,3220	B	8,2960	C	6,3780	D	14,4720	E	9,1940
4	A	5,8330	B	11,2760	C	12,8070	D	17,9600	E	15,4960
5	A	13,5100	B	9,1710	C	8,9180	D	9,0520	E	8,1240
6	A	11,2640	B	11,4630	C	4,2980	D	11,9490	E	11,2020
7	A	6,0120	B	8,7990	C	5,6620	D	12,7180	E	11,1230
8	A	12,8150	B	14,8180	C	7,2260	D	24,5460	E	13,6280
9	A	3,6750	B	7,8090	C	7,1450	D	10,0180	E	19,7390
10	A	10,6160	B	8,3270	C	6,7930	D	12,3450	E	14,8720
11	A	6,9490	B	9,9700	C	7,1740	D	15,6510	E	9,9290
12	A	17,3480	B	19,6610	C	17,7340	D	26,7790	E	23,4690
13	A	12,9130	B	22,1380	C	13,1280	D	29,8060	E	27,2680
14	A	17,9340	B	11,8870	C	6,7720	D	15,1040	E	9,3900
15	A	12,9110	B	11,4560	C	8,6870	D	13,1890	E	17,6120
16	A	7,9170	B	15,3570	C	13,6140	D	28,8790	E	13,6880
17*	A		B		C		D		E	
18	A	9,4240	B	9,3880	C	6,6420	D	17,3390	E	9,8620
Mean	A	10,40	B	12,22	C	9,03	D	17,66	E	14,41
SD	A	4,65	B	4,23	C	3,66	D	6,78	E	5,45
Min	A	11,469	B	7,809	C	4,298	D	9,052	E	8,124
Max	A	17,934	B	22,138	C	17,734	D	29,806	E	27,268

* Two subjects, No 2 and No 17, with baseline nicotine plasma levels exceeding 4 ng/ml were excluded from pharmacokinetic analysis.

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15.6 INDIVIDUAL PHARMACOKINETIC PARAMETERS.

T_{max} (min). N=16

Subj	Treat	T_{max}	Treat	T_{max}	Treat	T_{max}	Treat	T_{max}	Treat	T_{max}
1	A	120	B	30	C	24	D	30	E	30
2*	A		B		C		D		E	
3	A	60	B	30	C	24	D	24	E	30
4	A	90	B	24	C	30	D	30	E	30
5	A	90	B	24	C	24	D	45	E	45
6	A	240	B	24	C	30	D	30	E	30
7	A	90	B	30	C	16	D	16	E	24
8	A	120	B	30	C	24	D	24	E	30
9	A	90	B	8	C	30	D	45	E	16
10	A	120	B	30	C	24	D	30	E	30
11	A	90	B	45	C	16	D	30	E	45
12	A	24	B	16	C	24	D	45	E	24
13	A	120	B	24	C	30	D	30	E	30
14	A	90	B	30	C	30	D	16	E	16
15	A	120	B	45	C	90	D	90	E	90
16	A	120	B	24	C	30	D	24	E	30
17*	A		B		C		D		E	
18	A	120	B	16	C	24	D	24	E	16
Median		105		27		24		30		30
Min		24		8		16		16		16
Max		240		45		90		90		90

***** Two subjects, No 2 and No17, with baseline nicotine plasma levels exceeding 4ng/ml were excluded from pharmacokinetic analysis.

15.7 INDIVIDUAL PHARMACOKINETIC PARAMETERS.

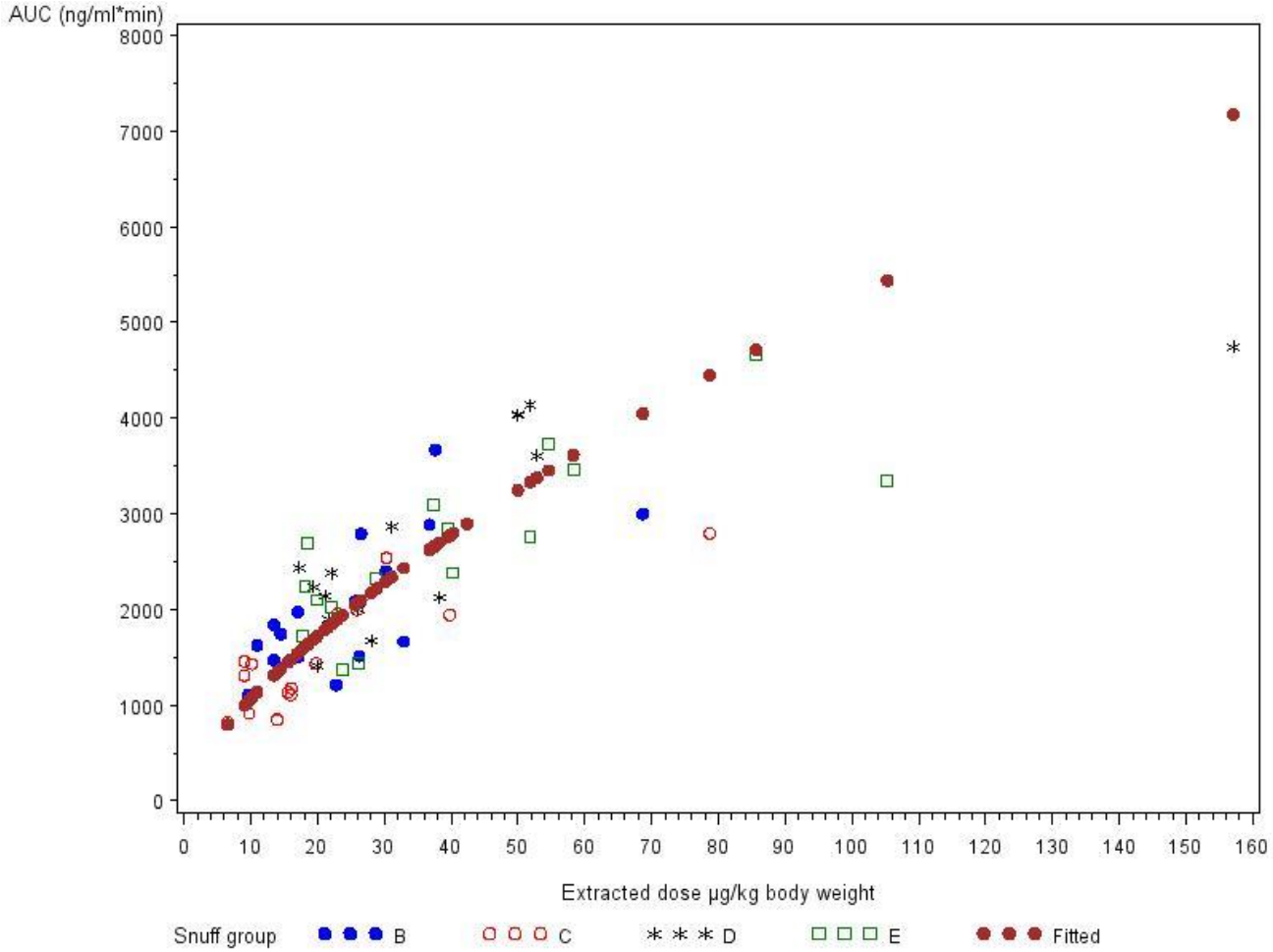
AUC_{inf} (µg/ml * min).

Subj	Tabl	AUC_{inf_obs}	Snus	AUC_{inf_obs}	Snus	AUC_{inf_obs}	Snus	AUC_{inf_obs}	Snus	AUC_{inf_obs}
1	A	4569.6594	B	2889.2142	C	1957.2076	D	4033.1646	E	3467.5988
2*	A	3804.259	B	2349.986	C	1757.232	D	3512.683	E	2935.997
3	A	447.2093	B	1108.7018	C	847.2729	D	1412.4616	E	1369.2264
4	A	1376.6176	B	2088.2206	C	1998.5254	D	2900.4722	E	3090.5300
5	A	3741.4279	B	1838.7592	C	1312.8609	D	1901.2043	E	2238.5489
6	A	5754.6947	B	1626.6265	C	915.4248	D	2440.9912	E	2102.0911
7	A	1794.6346	B	1471.1226	C	824.7121	D	2240.2905	E	2691.7573
8	A	3381.8082	B	2792.7787	C	1178.9698	D	2866.6012	E	2321.7111
9	A	984.5360	B	1513.7709	C	1137.2667	D	1675.5588	E	2386.7386
10	A	2718.5825	B	1515.9650	C	1107.8909	D	2128.1536	E	2855.0885
11	A	2117.7214	B	1975.4691	C	1459.9953	D	2142.0013	E	2027.9546
12	A	2903.3013	B	3001.8928	C	2797.7427	D	4749.2216	E	3351.1576
13	A	4690.3733	B	3671.9543	C	2540.7259	D	4042.5440	E	3731.5875
14	A	4788.1859	B	1746.7175	C	1431.9096	D	2385.4285	E	1724.4626
15	A	3526.7099	B	2400.7276	C	1947.3464	D	3611.5171	E	4663.7924
16	A	2624.0201	B	1667.0430	C	1437.9035	D	4141.7998	E	2764.3216
17*	A		B		C		D		E	
18	A	2201.7578	B	1213.4548	C	853.8294	D	2005.6110	E	1448.7183
Mean	A	2976.327	B	2032.65116	C	1484.349	D	2792.314	E	2639.705
SD	A	1491.838	B	724.533621	C	605.3012	D	1015.334	E	879.7015
Min	A	447.2093	B	1108.7018	C	824.7121	D	1412.462	E	1369.226
Max	A	5754.695	B	3671.9543	C	2797.743	D	4749.222	E	4663.792

***** Two subjects, No 2 and No17, with baseline nicotine plasma levels exceeding 4 ng/ml were excluded from pharmacokinetic analysis.

15.8 Dose proportionality of AUCinf (linear pharmacokinetics)

15.8.1 AUC_{inf} versus *In-vivo* extracted dose per kg Body Weight with fitted curve



Legend. The dose proportionality was examined by comparison of the relationship between the AUC_{inf} and the extracted dose per kg body weight. As seen above the plot showed a fairly linear relationship.

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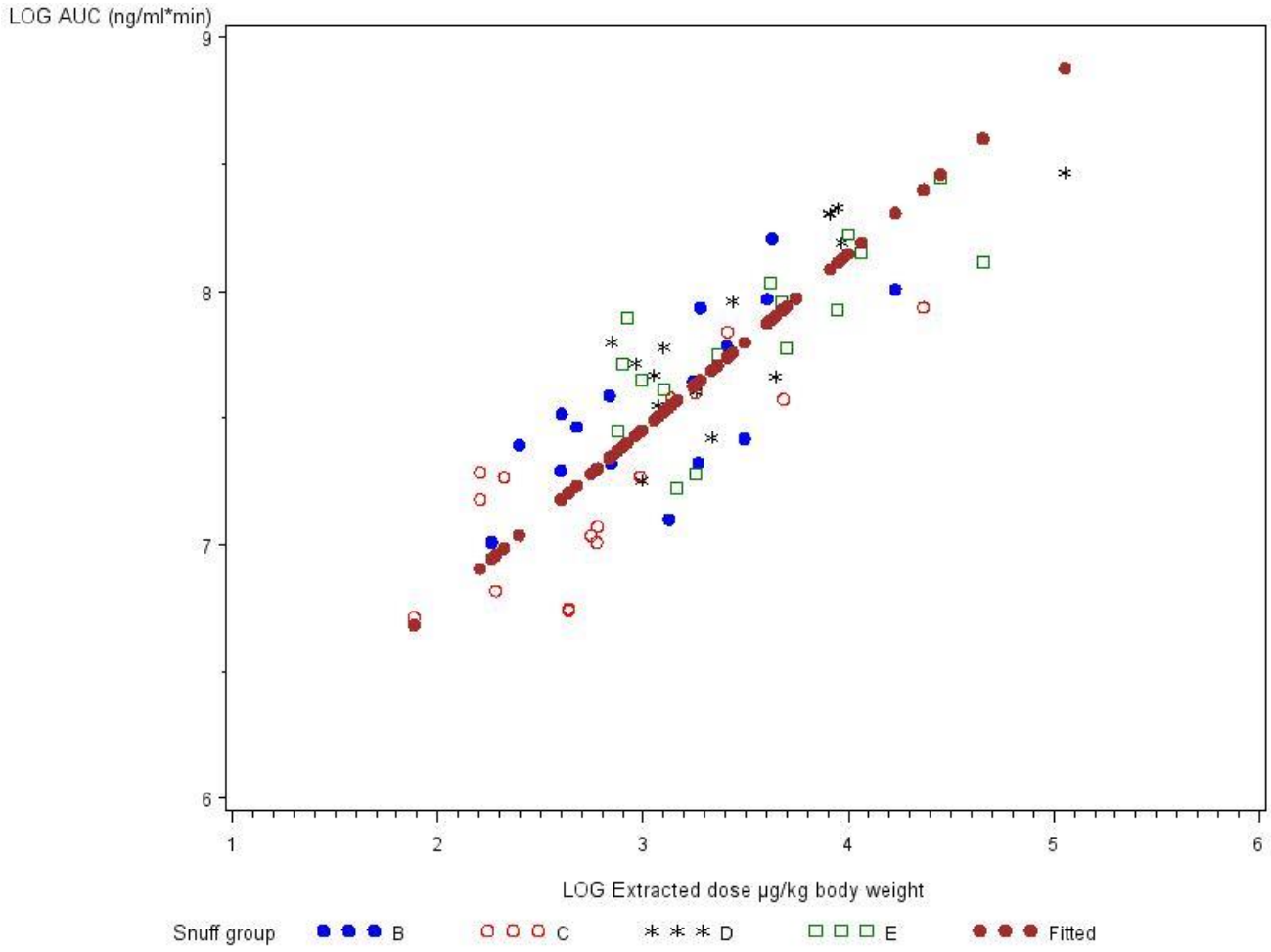
15.8.2 Log AUC_{inf} versus Log In-vivo Extracted dose/kg Body Weight with fitted line



Legend. The dose proportionality was examined by comparison of the relationship between the AUC_{inf} and the extracted dose per kg body weight. As seen above even better linearity was achieved when the relationship was plotted in a logarithmic X-Y diagram.

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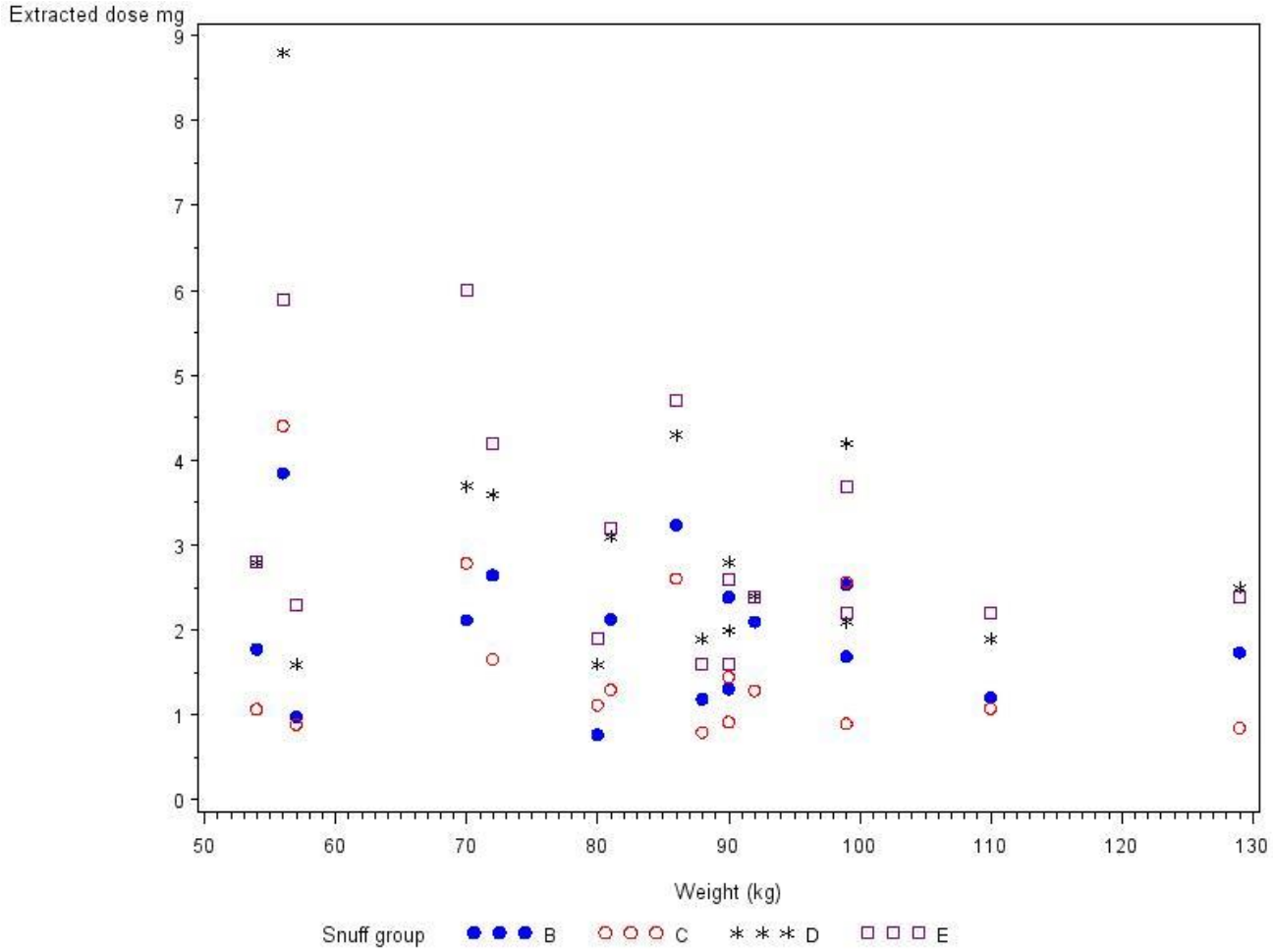
15.8.3 *In-vivo* extracted dose versus kg Body Weight



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16 VAS RECORDINGS OF SUBJECTIVE EFFECTS

Mean Head Rush

Time (Min)	A - Tablet	Snus- B	Snus - C	Snus - D	Snus - E
0	2,21	1	1,5	1,73	1,47
2	2	2,63	1,94	2,2	2
4	2,73	2,81	2,94	2,93	2,75
8	2,73	2,81	3,38	3,87	3,69
16	3	2,93	3,31	3,73	3,88
24	3,07	3,2	3,19	3,73	3,81
30	3,08	3,06	2,75	3,53	3,88

Mean Craving/Urges to smoke

Time (Min)	A - Tablet	Snus- B	Snus - C	Snus - D	Snus - E
0	4,21	4,38	4,81	4,93	4,33
2	4,14	3,06	3,94	3,67	4,13
4	3,87	2,88	2,94	3,2	3,38
8	3,2	2,63	2,13	2,13	2,19
16	3,21	2,33	1,94	1,8	1,19
24	2,67	1,8	2	1,4	1,13
30	2,38	1,75	1,63	1,47	1

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17 VAS RECORDINGS OF ADVERSE EFFECTS

Mean Salivation

Time (Min)	A - Tablet	Snus- B	Snus - C	Snus - D	Snus - E
0	1,92	0,94	0,88	1,47	1,07
2	1,87	1,56	1,25	1,53	1,44
4	2	1,56	1,75	1,60	1,75
8	2,07	1,63	1,69	1,60	1,81
16	1,71	1,80	1,69	1,87	2
24	1,60	1,60	1,56	1,80	2
30	1,31	1,63	1,81	1,80	2,25

Mean Mouth/Throat burn

Time (Min)	A - Tablet	Snus- B	Snus - C	Snus - D	Snus - E
0	0,33	0,75	0,94	1,40	1,13
2	0,60	1,06	1,07	1,60	0,88
4	0,73	1,19	1,13	1,67	1,31
8	0,67	1,19	1,56	1,40	1,56
16	1,00	1,20	1,31	1,53	1,19
24	0,93	1,33	1,19	1,60	1,50
30	0,85	1,31	1,31	1,60	1,75

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18 STATISTICAL ANALYSIS OF HEAD RUSH VAS RATINGS

Table 1 Wilcoxon signed rank test on head rush VAS score by time point

Comparison	**minute**	**p - value**
Treatment A vs treatment B	8	1.0000
	16	0.3633
	24	0.9570
	30	0.8838
Treatment A vs treatment C	8	0.1523
	16	0.8208
	24	1.0000
	30	0.6733
Treatment A vs treatment D	8	0.1250
	16	0.8887
	24	0.6523
	30	0.7031
Treatment A vs treatment E	8	0.1143
	16	0.2832
	24	0.4844
	30	0.3672
Treatment B vs treatment C	8	0.1250
	16	0.4082
	24	0.9414
	30	0.5020
Treatment B vs treatment D	8	0.0898
	16	0.1172
	24	0.3320
	30	0.5039
Treatment B vs treatment E	8	0.0439
	16	0.0225
	24	0.0742
	30	0.1484
Treatment C vs treatment D	8	0.7402
	16	0.5576
	24	0.1875
	30	0.0859

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Comparison	**minute**	**p - value**
Treatment C vs treatment E	8	0.7471
	16	0.2363
	24	0.1250
	30	0.0232
Treatment D vs treatment E	8	0.8870
	16	0.6387
	24	0.3984
	30	0.0742

Table 2 Summary statistics for head rush VAS score, by time point and treatment

		VAS score								
		n	Mean	SD	SEM	Min	Q1	Median	Q3	Max
Minute	Treatment									
8	A	15	2.7	2.3	0.6	0	1.0	2.0	5.0	7
	B	16	2.8	2.4	0.6	0	1.0	2.0	4.5	8
	C	16	3.4	2.4	0.6	0	2.0	3.0	4.5	9
	D	15	3.9	3.0	0.8	1	1.0	2.0	7.0	10
	E	16	3.7	2.2	0.6	0	2.0	3.5	5.0	8
16	A	15	3.2	2.4	0.6	0	1.0	3.0	5.0	8
	B	16	2.9	2.5	0.6	0	1.5	2.0	4.0	8
	C	16	3.3	2.6	0.6	0	2.0	2.5	5.0	8
	D	15	3.7	3.0	0.8	0	2.0	2.0	6.0	10
	E	16	3.9	2.3	0.6	0	2.5	3.0	6.0	8
24	A	15	3.1	2.2	0.6	0	1.0	3.0	5.0	7
	B	16	3.1	2.8	0.7	0	1.0	2.0	4.5	9
	C	16	3.2	2.6	0.7	0	1.0	3.0	4.0	9
	D	15	3.7	3.0	0.8	0	1.0	4.0	5.0	10
	E	16	3.8	2.8	0.7	0	2.0	3.0	5.0	10
30	A	15	2.9	2.1	0.5	0	1.0	3.0	4.0	7
	B	16	3.1	2.6	0.6	0	1.0	2.5	3.5	9
	C	16	2.8	2.7	0.7	0	1.0	2.0	3.5	10
	D	15	3.5	3.0	0.8	0	1.0	4.0	5.0	10
	E	16	3.9	3.1	0.8	0	1.5	3.0	6.0	10

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Table 3 Summary statistics for change in head rush VAS score between different treatments, by time point

		Change in VAS score								
		n	Mean	SD	SEM	Min	Q1	Median	Q3	Max
Treatment comparison	Minute									
A vs B	8	15	0.0	1.3	0.3	-2	-1.0	0.0	1.0	2
	16	15	0.3	1.4	0.4	-2	-1.0	0.0	2.0	3
	24	15	0.1	1.4	0.4	-3	-1.0	0.0	1.0	3
	30	15	-0.1	1.4	0.4	-3	-1.0	0.0	1.0	2
A vs C	8	15	-0.7	1.7	0.4	-3	-2.0	0.0	0.0	3
	16	15	-0.1	2.1	0.5	-4	-1.0	0.0	1.0	3
	24	15	-0.1	2.0	0.5	-4	-1.0	0.0	1.0	4
	30	15	0.2	2.1	0.5	-4	-1.0	1.0	2.0	3
A vs D	8	14	-0.8	1.8	0.5	-4	-1.0	-1.0	0.0	3
	16	14	-0.2	2.1	0.6	-5	-1.0	0.0	1.0	3
	24	14	-0.4	2.3	0.6	-5	-1.0	0.0	1.0	4
	30	14	-0.3	2.1	0.6	-4	-2.0	0.0	1.0	3
A vs E	8	15	-0.7	1.4	0.4	-3	-2.0	-1.0	0.0	2
	16	15	-0.4	1.2	0.3	-2	-1.0	0.0	0.0	2
	24	15	-0.5	2.2	0.6	-5	-1.0	0.0	0.0	3
	30	15	-0.7	2.2	0.6	-6	-2.0	0.0	1.0	2
B vs C	8	16	-0.6	1.2	0.3	-3	-1.0	-0.5	0.0	2
	16	16	-0.4	1.3	0.3	-4	-1.0	0.0	0.5	1
	24	16	-0.1	1.2	0.3	-3	-0.5	0.0	1.0	2
	30	16	0.3	1.3	0.3	-2	0.0	1.0	1.0	2
B vs D	8	15	-0.9	1.7	0.4	-5	-1.0	0.0	0.0	1
	16	15	-0.6	1.4	0.4	-4	-2.0	0.0	0.0	1
	24	15	-0.4	1.3	0.3	-3	-1.0	0.0	0.0	2
	30	15	-0.3	1.6	0.4	-3	-2.0	0.0	1.0	3
B vs E	8	16	-0.9	1.5	0.4	-4	-1.5	-1.0	0.0	2
	16	16	-0.9	1.5	0.4	-4	-2.0	-1.0	0.0	1
	24	16	-0.7	1.5	0.4	-4	-1.5	-0.5	0.0	3
	30	16	-0.8	1.9	0.5	-5	-1.5	0.0	0.0	2
C vs D	8	15	-0.3	2.3	0.6	-4	-1.0	0.0	2.0	3
	16	15	-0.2	1.3	0.3	-2	-1.0	0.0	1.0	2
	24	15	-0.4	0.9	0.2	-3	-1.0	0.0	0.0	1

Date: 2013-06-17

		Change in VAS score								
		n	Mean	SD	SEM	Min	Q1	Median	Q3	Max
	30	15	-0.7	1.2	0.3	-3	-2.0	0.0	0.0	2
C vs E	8	16	-0.3	1.9	0.5	-6	-1.0	0.0	1.0	2
	16	16	-0.6	1.8	0.5	-5	-2.0	0.0	1.0	2
	24	16	-0.6	1.4	0.3	-4	-1.0	-0.5	0.0	2
	30	16	-1.1	1.7	0.4	-5	-1.5	-1.0	0.0	1
D vs E	8	15	0.1	2.1	0.5	-3	-1.0	-1.0	1.0	5
	16	15	-0.3	1.6	0.4	-3	-1.0	0.0	0.0	3
	24	15	-0.3	0.9	0.2	-2	-1.0	0.0	0.0	1
	30	15	-0.6	1.1	0.3	-3	-1.0	-1.0	0.0	1