Cytisine concentration-effect relationships in human smokers

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Abstract

Cytisine is a plant alkaloid that is a partial agonist for the $\alpha_4\beta_2$-nicotinic acetylcholine receptor and is used as a smoking cessation medication (Tabex®). Double-blind, randomised, placebo-controlled trials show that cytisine is more effective than placebo in achieving long-term, continuous abstinence from smoking. At the start of this PhD there was no published information on the pharmacokinetics and metabolism of cytisine in humans or indeed, whether there is any relationship between cytisine exposure and effect.

The main aims of this thesis were therefore: to obtain basic pharmacokinetic data for cytisine in humans, to study the effects of cytisine on physiological and psychological measures in smokers and to explore whether these effects could be related to the plasma concentrations of cytisine in human smokers who were instructed to adhere to the standard dosing regimen of Tabex®

In order to study the human metabolism and pharmacokinetics of cytisine, a sensitive analytical method using mass spectrometry (LC-MS) was developed and validated. This method was used to support the subsequent pharmacokinetic studies. In the first study, seven participants took a single dose (3 mg) of Tabex® and blood samples were collected at various times up to 24 hours. Cytisine plasma concentrations were measured. In the second study, another set of participants (n=11) took Tabex® using the standard 25-day dosing regimen recommended by the manufacturer. Blood samples were collected and cigarette craving, withdrawal, mood and smoking satisfaction were measured using self-report methods validated in the literature.

Following a single dose administration, cytisine peak plasma concentrations typically occurred at 2 hours. Following this, cytisine concentrations declined in a monophasic manner with a mean half-life of 4.8 hours. No metabolites were detected. In the second study, accumulation of cytisine in plasma was observed on day 1. However, with the recommended dosing regimen, cytisine does not reach steady state concentration in plasma. There was also large between-subject variability in cytisine pharmacokinetics. Cytisine appeared to reduce cigarette cravings, but there did not appear to be a simple relationship between craving and cytisine plasma concentration.

In summary, this thesis presents the first reported human cytisine pharmacokinetic data. The information gained from these studies may be used to inform the design of future trials that explore different dosing regimens of cytisine.

Dedication

This thesis is dedicated to my parents who have supported me all the way since the beginning of my studies.

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Glossary

$\alpha_4\beta_2$-nAChR	alpha-4-beta-2-nicotinic acetylcholine receptor
ACN	acetonitrile
CI	confidence Interval
CL	clearance
C_{max}	peak concentration
CNS	central nervous system
CO	carbon monoxide
CYP	cytochrome P450
DBP	diastolic Blood Pressure
DDI	drug-drug interaction
EC	electronic cigarette
FTND	Fagerström Test for Nicotine Dependence
HPLC	high performance liquid chromatography
HR	heart rate
HSI	Heaviness of Smoking Index
IS	internal standard
LC-MS	liquid chromatography-mass spectrometry
LOQ	limit of quantification
m/z	mass/charge
mCEQ	modified Cigarette Evaluation Questionnaire
MeOH	methanol
MPSS	Mood and Physical Symptoms Scale
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NRT	nicotine replacement therapy
PD	pharmacodynamics
PK	pharmacokinetics
POMS	Profile Of Mood States
QC	quality control
QSU-brief	Questionnaire on Smoking Urges-brief
RR	relative risk
SBP	systolic Blood Pressure

SIM	selective ion monitoring
TIC	total ion current
T_{max}	time taken to reach peak concentration
TMD	total mood disturbance
UDPGA	uridine 5’-diphosphoglucuronic acid
UGT	UDP glucuronyl transferase
UV	ultraviolet
V_D	volume of distribution

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CO-AUTHORS

Name	Nature of Contribution
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Dr Janie Sheridan	Study design, supervision of student, contribution to editing and writing of paper
Dr David Newcombe	Input regarding questionnaires utilised in project, supervision, contibution to editing paper

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
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Chapter 1 General Introduction

1.1 Introduction and overview

Tobacco smoking is a major cause of many diseases including cancers, cardiovascular disease and respiratory diseases (U.S. Department of Health and Human Services 2004, Jha 2009). Every year smoking contributes to the death of nearly 6 million people world-wide (World Health Organization 2015). In New Zealand, 4500 to 5000 people each year die due to smoking-related diseases (Ministry of Health 2010, Ministry of Health 2014). In 2013/2014, 17.2% of the population aged 15 years and over were current smokers (smoke at least monthly) in New Zealand (Ministry of Health 2014). The overall smoking rate has slowly declined over the last decade (20% of the general population were current smokers in 2006/2007) but this decline is not equally distributed among all ethnic groups. Smoking rates remain the highest amongst Māori (39.5%) and this rate is approximately 1.4 times higher than Pacific (28.3%) and more than double that of European (16.2%) and Asian (14.4%) ethnic groups (Ministry of Health 2014). Smoking contributes significantly to inequalities in health and it is estimated that approximately 10% of the discrepancies in health between Māori and non-Māori are attributed to smoking (Blakely T, Fawcett J et al. 2006).

Approximately 1.7 billion New Zealand dollars are spent each year on healthcare to treat the consequences of nicotine dependence and other diseases associated with smoking (O'Dea and Thomson 2007). Smoking-related health problems are thus a significant burden to healthcare providers and impose a considerable burden on the limited healthcare funding and resources. In 2011, the New Zealand Government adopted the Smokefree 2025 goal for New Zealand which aims to reduce the overall prevalence of adult smoking of less than 5% for all populations (Wilson, Blakely et al. 2011). Smoking cessation is, therefore, one of the key health priorities for the New Zealand government (Ministry of Health 2013) and rigorous research is required to explore effective treatments and interventions for smoking cessation.

Several approaches to help smokers quit are currently available, including non-pharmacological (behavioural therapy) and pharmacological interventions. Although behavioural counselling (Lancaster and Stead 2005) or physician's advice (Stead, Bergson et al. 2008) may help smokers to quit smoking, only 3 to 5% of smokers who try to quit smoking without the use of medication succeed, as determined by abstinence at 6 months (Hughes, Keely et al. 2004). Nicotine has an important role in smoking (Balfour 2004, Benowitz 2008) and therefore pharmacological treatments

are important as they may address this component (Benowitz 2010). The combination of medication and counselling has been shown to be more effective than either alone (Asfar, Ebbert et al. 2011).

This chapter presents a brief overview of nicotine and important measures in smoking cessation. It will also review the current smoking cessation interventions with a focus on pharmacological medications. The aims and outline of the thesis are outlined at the end of this chapter.

1.2 Nicotine

Tobacco smoke contains over 4000 chemical components including nicotine, carbon monoxide, aromatic hydrocarbons and N-nitrosamines, some of which are known carcinogens (Hecht 1999). Of these, nicotine is regarded as the main pharmacological driver of smoking behaviour (Stolerman and Shoaib 1991, Balfour, Wright et al. 2000, Di Chiara 2000), although other components in tobacco smoke may also contribute to addiction to smoking (Green, Crooks et al. 2001, Rose 2007). Understanding the neuropharmacology of nicotine and its role in smoking has greatly contributed to the development of pharmacotherapies for smoking cessation.

1.2.1 Nicotine dependence and withdrawal

The pharmacology of nicotine and the neurobiology of nicotine dependence have been reviewed extensively in the literature (Benowitz 1990, Corrigall, Franklin et al. 1992, Di Chiara 2000, Tapper, McKinney et al. 2004, Benowitz 2008, Benowitz 2008, Cosci, Corlando et al. 2009, Govind, Vezina et al. 2009). It is widely accepted that nicotine dependence plays a crucial role in maintaining smoking behaviour (Corrigall, Franklin et al. 1992, Cosci, Corlando et al. 2009, Benowitz 2010). Nicotine's actions at nicotinic acetylcholine receptors (nAChRs) are thought to play a vital role in neurological mechanisms that lead to nicotine dependence, tolerance and withdrawal (Benowitz 2008, Benowitz 2010). The most abundant nAChRs in the human brain are the $\alpha_4\beta_2$, $\alpha_3\beta_4$ and α_7 subtypes (Paterson and Nordberg 2000). Of these, the $\alpha_4\beta_2$ subtype is the most abundant in the human brain and is regarded as the main receptor that mediates nicotine dependence (Paterson and Nordberg 2000, Tapper, McKinney et al. 2004). The binding of nicotine to these receptors leads to a release of various neurochemicals in the brain such as dopamine, norepinephrine and serotonin (Benowitz 2008). Many of these neurochemicals are implicated in mood modulation and resemble those released with antidepressant drugs (Stahl, Pradko et al. 2004). Of these neurotransmitters,

dopamine has received much attention because of its role in reward (Spanagel and Weiss 1999, Berke and Hyman 2000). An important dopaminergic pathway is the mesolimbic pathway which comprises of neurons in the ventral tegmental area (VTA) of the midbrain that projects to the nucleus accumbens as well as other areas in the forebrain such as the prefrontal cortex, the amygdala and the striatum (Pidoplichko, Noguchi et al. 2004). Studies have shown nicotine increases dopamine in the nucleus accumbens which reinforces nicotine administration (Corrigall, Franklin et al. 1992, Balfour, Wright et al. 2000, Di Chiara 2000). In animal studies, blocking dopamine release in the nucleus accumbens reduced nicotine self-administration indicating that it decreased the rewarding effects of nicotine (Stolerman and Shoaib 1991, Corrigall, Franklin et al. 1992). Repeated use of nicotine leads to neuroadaptive changes such as receptor desensitisation, inactivation and increased expression of nicotinic receptors (up-regulation) in the brain (Govind, Vezina et al. 2009). This can lead to the development of tolerance such that the smoker requires an increased dose of nicotine to achieve the same level of reward from smoking (Hukkanen, Jacob et al. 2005).

Cigarette smoking is motivated by the positive reinforcing effects of nicotine such as mood modulation, increased vigilance, reduced stress and relaxation (Pomerleau and Pomerleau 1992, Benowitz 1996) as well as negative reinforcement which lead the smoker to continue smoking in order to relieve nicotine withdrawal symptoms. Nicotine withdrawal can include somatic symptoms (e.g. bradycardia and gastrointestinal discomfort) and affective symptoms such as irritability, anxiety, poor concentration, depressed mood and cravings for cigarettes (American Psychiatric Association, 1994; Hughes et al., 1991). These affective symptoms have been shown to have an important role in sustaining nicotine dependence (Koob, Markou et al. 1993, Markou, Kosten et al. 1998) and current smoking cessation medications are used to reduce or relieve some of these symptoms in an abstaining smoker during a quit attempt. In particular, craving, a strong intense desire or urge (to smoke), is a prominent characteristic of nicotine withdrawal (Hughes and Hatsukami 1986, Gritz, Carr et al. 1991, Shiffman, West et al. 2004).

1.2.2 Importance of route of delivery

The route of nicotine administration plays an important role in the development of nicotine dependence. Inhalation of cigarette smoke rapidly delivers nicotine to the brain from the arterial circulation, the peak concentration (C_{max}) is reached within 20 seconds (Zevin, Gourlay et al. 1998). The short delay between inhaling nicotine from cigarette smoking and its delivery to the brain (and hence the resulting central nervous system effects) allows the smoker to titrate the dose of nicotine to obtain a desired pharmacological effect. Smoking cigarettes leads to relatively high arterial blood

concentrations of nicotine (20-60 ng/mL) (Benowitz, Kuyt et al. 1982, Henningfield and Keenan 1993, Gourlay and Benowitz 1997). Cigarette smoking and intravenous nicotine are thus highly reinforcing (Pickworth, Bunker et al. 1994).

In contrast, other routes of delivery lead to slower absorption and lower C_{max} of nicotine than that achieved with cigarette smoking. After oral administration, nicotine undergoes extensive first-pass metabolism with a low bioavailability (between 20 and 45%) (Benowitz, Jacob et al. 1991, Compton, Sandborn et al. 1997). Nicotine can also be delivered via transdermal systems (skin patch) which offer the slowest delivery of nicotine; the lag time between nicotine administration and the time that it appears in the blood is around 1 hour (Fant, Henningfield et al. 2000). Blood nicotine concentration increases slowly for 6 to 10 hours and a plateau is maintained for 7 to 8 hours (Benowitz 1995). Whilst cigarette smoking and transdermal delivery may yield the same delivery of nicotine, the C_{max} is much lower following transdermal delivery (Benowitz 1995). The use of transdermal nicotine produces little (if any) behavioural reinforcement (Henningfield and Keenan 1993).

Nicotine absorption via oral mucosa (i.e. by chewing nicotine gum) is faster than transdermal delivery, but relatively slow compared with cigarette smoking, and nicotine concentrations reaches steady state at 30 minutes (Benowitz, Jacob et al. 1987). Nicotine extraction from chewing is incomplete and dose-dependent (53% extraction in 2 mg gum and 72% extraction in 4 mg gum). Nicotine swallowed while chewing gum is subject to first-pass metabolism (Benowitz, Jacob et al. 1987). Like transdermal delivery, chewing nicotine gum results in lower nicotine plasma concentrations compared to smoking (McNabb 1984, Benowitz, Jacob et al. 1987).

In contrast to transdermal or oral administration, nicotine delivery via nasal mucosa (i.e. nasal sprays) can deliver nicotine quickly to the brain (Benowitz, Kuyt et al. 1982). Peak plasma concentrations are reached after 5 minutes post dosing (Sutherland, Stapleton et al. 1992, Schneider, Lunell et al. 1996), but results in lower C_{max} nicotine concentrations than smoking (Gourlay and Benowitz 1997). Nicotine doses, however, can be repeated frequently with nasal sprays, although nasal irritation often constrains repeated intake (Sutherland, Stapleton et al. 1992). When the abuse liability of nicotine containing products in smoking cessation was compared, the overall abuse liability was reported to be low for all products (including nasal sprays) (West, Hajek et al. 2000).

In summary, the route and rate of nicotine dose depend on the delivery system which plays a critical role in the development of nicotine dependence.

1.2.3 Cotinine

Nicotine undergoes rapid and extensive hepatic metabolism by the cytochrome P450 (CYP) family, in particular by the CYP2A6 subtype and to a lesser extent by CYP2B6 and CYP2E1 (Hukkanen, Jacob et al. 2005). The primary metabolite of nicotine is cotinine (Dempsey, Tutka et al. 2004). Cotinine has a longer half-life than nicotine (16 h vs 2 h) (Benowitz and Jacob 1994, Benowitz, Jacob et al. 1995). Due to its long half-life, cotinine concentrations have been used as biomarkers of nicotine exposure (Marrone, Paulpillai et al. 2010) and cotinine (in blood, urine or saliva) is often measured in smoking cessation trials for biochemical validation (also called biochemical verification), the procedure of checking whether a person has or has not smoked (or used tobacco).

Studies on the behavioural effects of cotinine administration have found conflicting results. In an uncontrolled study (Benowitz, Kuyt et al. 1983), there was some relief of nicotine withdrawal symptoms and reduced tension (measured by Profile of Mood States (POMS)) in abstinent smokers during cotinine intravenous infusion, whilst another study found a 30 mg dose of cotinine increased the mood scores for restlessness and anxiety (Keenan, Hatsukami et al. 1994). On the other hand, it has been reported that no effects were observed on nicotine withdrawal in smokers who received oral cotinine in doses up to 160 mg (Hatsukami, Grillo et al. 1997). A subsequent study (Hatsukami, Pentel et al. 1998) reported that a dose of cotinine (80 mg) resulted in approximately three-fold higher plasma cotinine concentrations than that found in average smokers, and did not produce any effect on nicotine withdrawal symptoms. In a double-blind, placebo-controlled study, no differences were found in subjective measures such as mood (measured by POMS) between placebo and cotinine groups (Zevin, Jacob et al. 2000)

1.3 Measurements in smoking cessation

As previously mentioned, current pharmacological interventions used for smoking cessation aim to reduce the impact of nicotine dependence by relieving negative symptoms experienced during a quit attempt. Pharmacological interventions may, therefore, help smokers to cope with the behavioural and psychological aspects of smoking. It is important to assess nicotine withdrawal symptoms when studying the effects of smoking cessation drugs because they are common physiological and psychological symptoms experienced during smoking cessation and may play an important role in promoting relapse (Killen and Fortmann 1997, Baker, Piper et al. 2004, Donny, Griffin et al. 2008). Craving is regarded as the most important withdrawal symptom because the severity of craving has

been shown to be associated with the heaviness of smoking (Goedeker and Tiffany 2008) and may also predict satisfaction from smoking (Shiffman and Kirchner 2009). Research has shown that the level of craving may predict future success of quitting (Killen and Fortmann 1997, Ferguson, Shiffman et al. 2006, McCarthy, Piasecki et al. 2006, Bailey, Harrison et al. 2009). On the other hand, some studies have shown that the existence of craving and other withdrawal symptoms do not predict or promote relapse to smoking (Hughes 1993, Hughes, Higgins et al. 1994); however, it is generally accepted that withdrawal and craving are clinically important because these symptoms can cause discomfort which may cause a smoker to fail their quit attempt (Shiffman, West et al. 2004).

The Diagnostic and Statistical Manual (DSM) of Mental Disorders is the standard classification of mental disorders including substance use disorders. The nicotine withdrawal symptoms listed in the fourth version of the DSM (DSM-IV) included irritability, restlessness, insomnia, anxiety, depression, increase appetite, sleep disturbance and poor concentration (American Psychiatric Association 2000). Craving has been included in the most recent version, DSM-5 (Baker, Breslau et al. 2012). These symptoms have also been reviewed by experts in the field (Shiffman, West et al. 2004).

1.3.1 Rating scales

Rating scales, often in the form of questionnaires, are used widely to measure craving and withdrawal. A questionnaire that specifically assesses cigarette craving is the Questionnaire on Smoking Urges-Brief (QSU-brief) (Cox, Tiffany et al. 2001), a shorter version of the original, the Questionnaire on Smoking Urges (QSU) (Tiffany and Drobes 1991). This questionnaire is extensively used in the literature (Teneggi, Tiffany et al. 2002, Nakamura, Oshima et al. 2007, Tsai, Cho et al. 2007). There are also validated questionnaires used to measure other withdrawal symptoms including the Mood and Physical Symptoms Scale (MPSS) (West and Hajek 2004), the Minnesota Nicotine Withdrawal Scale (MNWS) (Hughes and Hatsukami 1986), the Shiffman Scale (SS) (Shiffman, Khayrallah et al. 2000), the Wisconsin Smoking Withdrawal Scale (WSWS) (Welsch, Smith et al. 1999) and the Cigarette Withdrawal Scale (CWS) (Etter 2005). These scales differ in rating style and scales, wording of items and length of scale. For example, the MPSS, the MNWS, the WSWS and the CWS all use a 5-point response scale for each symptom, whereas the SS uses a 10-point scale. In addition, the wording of the response scales differs for each questionnaire to describe the severity of the feeling/symptom assessed. For example, the response scale in the MPSS consists of: *not at all, slightly, somewhat, very, extremely* while the 5-point scale in the MNWS is: *none, slight, mild, moderate, severe,* the WSWS use a more indirect *disagree-agree* scale: *strongly disagree, disagree,*

neutral, agree, strongly agree and the SS labels just the extremes of the response scale (10 point-scale: low to high). The words to describe each symptom may also differ, for example, the MPSS uses a single word for each withdrawal symptom (*irritable, restless, depressed, anxious,* etc.) whereas the MNWS uses more than one word for each item measured (e.g. *angry, irritable, frustrated* is one item, *anxious/nervous* is one item). The SS and CWS use more than one item to measure each withdrawal symptom (e.g. the SS uses *depressed, sad, blue* and *miserable* as separate items to measure depression). The WSWS and the CWS do not measure restlessness. Regardless of these differences, these scales have been shown to have a good reliability and sensitivity to abstinence and are useful in assessing total withdrawal discomfort (West, Ussher et al. 2006, West and Ussher 2010).

The Fagerström Test for Nicotine Dependence (FTND) (Heatherton, Kozlowski et al. 1989) is the most widely used scale for determining the severity of nicotine dependence. The Heaviness of Smoking Index (HSI) (Borland, Yong et al. 2010) consists of two questions from the FTND and is also routinely used (Borland, Yong et al. 2010). Both assessments can identify smokers with high dependence with high specificity (Perez-Rios, Santiago-Perez et al. 2009). The modified Cigarette Evaluation Questionnaire (mCEQ) (Cappelleri, Bushmakin et al. 2007) is also a widely used scale used to assess satisfaction with smoking in smokers who receive an intervention for smoking cessation (Nakamura, Oshima et al. 2007, Tsai, Cho et al. 2007, Walker, Howe et al. 2014). The Profile of Mood States (POMS) is validated questionnaire widely used in previous clinical trials to measure mood states including tension, depression, anger, fatigue, vigour, confusion and total mood disturbance (McNair, Lorr et al. 1971).

1.3.2 Choice of questionnaires for this thesis

Currently, researchers use a variety of validated scales to measure subjective measures.

During the planning of the PhD, a cytisine non-inferiority smoking cessation trial (Cytisine as a smoking cessation aid (CASCAID) study) (Walker, Howe et al. 2011) was being conducted by a research team at the University of Auckland, and Dr Walker was recruited to be an advisor for the PhD. The study used the Fagerström Test for Nicotine Dependence (FTND) to determine level of nicotine dependence in smokers, the Mood and Physical Symptoms Scale (MPSS) to measure withdrawal and the modified Cigarette Evaluation Questionnaire (mCEQ) to measure smoking satisfaction. For consistency, the studies in this thesis used the same questionnaires as those used in the CASCAID trial. In addition, the Questionnaire on Smoking Urges-brief (QSU-brief) (Cox, Tiffany et al. 2001) was used to measure cigarette craving and the Profile Of Mood States (POMS) (McNair,

Lorr et al. 1971) was used to measure mood. These two questionnaires were selected as they have been used extensively in previous large studies. Questionnaires used in this study were chosen after discussions with Dr Walker. Details of these questionnaires are described in Chapters 4 and 5.

1.4 Smoking cessation therapies

Prior to the 1970s, when nicotine replacement therapy (NRT) was first introduced, behavioural treatments were the only methods available to aid smoking cessation (Goodman 2004). NRT was the only pharmacological treatment approved for this purpose until the antidepressant bupropion was approved by the US Food and Drug Administration (FDA) in 1997. Both NRT and bupropion remain first line pharmacotherapy for smoking cessation (Cahill, Stevens et al. 2014). Nortriptyline is another antidepressant medication that may be used as a second line pharmacotherapy for smoking cessation (Hughes, Stead et al. 2005). Clonidine, a medication used for hypertension, has been shown to have some effect for smoking cessation and is proposed as second-line pharmacotherapy (Gourlay, Stead et al. 2004). Varenicline is the first non-nicotine pharmacotherapy developed to specifically target nicotinic receptors in order to counteract the effects of nicotine dependence (Foulds 2006). Varenicline is also a first line pharmacotherapy in helping smokers quit (Cahill, Stevens et al. 2014). All of these medications are more effective than placebo in helping smokers to quit (pooled RRs from Cochrane Reviews in Table 1.1). Electronic cigarettes are devices that can deliver vapourised nicotine, but they are not licenced as smoking cessation therapies, and there are limited data on the effectiveness of using these devices. These medications are discussed below in the order of their development, as this highlights the shift of the research paradigm in pharmacotherapy for smoking cessation. A separate section follows on cytisine.

1.4.1 Nicotine Replacement Therapy

NRT is the most common medication used in smoking cessation. The development of nicotine dependence is believed to be mediated mainly via nicotine's agonistic actions at *α4β2*-nAChRs which stimulates the release of dopamine (Coe, Brooks et al. 2005). NRT replaces the source of nicotine and may reduce unwanted symptoms of withdrawal in abstinent smokers. It aids smoking cessation by reducing nicotine withdrawal symptoms and craving that are experienced by an abstinent smoker (Shiffman, Ferguson et al. 2006). Therefore, NRT increases the chances of long term abstinence by reducing motivation to smoke and addressing the physiological withdrawal symptoms that reinforce

the smoker to continue to smoking (West and Shiffman 2001). NRT is available in several formulations such as gum, inhaler, lozenge, nasal spray and transdermal patch. A 2008 study reported the pooled relative risk (RR) of abstinence of NRT (gum, patch, inhaler, lozenges and nasal spray) compared to placebo or non-NRT control group to be 1.58 (95% confidence interval (CI) 1.53 to 1.66) (Stead, Perera et al. 2008). Most of the conventional NRTs do not relieve withdrawal symptoms completely since they do not reproduce the rapid and high exposure of nicotine such as that produced with cigarette smoking (see Section 1.2.2) (Benowitz 1993).

Although NRT is considered safe for most smokers, one of the limitations of this treatment is the issue with treatment acceptability. Some people do not favour using nicotine to treat their nicotine dependence, preferring a treatment that does not contain nicotine (Etter and Perneger 2001, Mooney, Leventhal et al. 2006). Also, NRT may not be effective in some smokers and may cause adverse effects such as local irritation (skin patches), nausea, vomiting and insomnia which can limit their use (Caldwell, Burgess et al. 2010). Therefore more effective and safer treatments are needed to aid smoking cessation.

1.4.2 Antidepressant medications

Antidepressant medications have been an important area of smoking cessation research. Although the mechanism(s) of action underlying smoking cessation are unclear, there are several reasons for using antidepressants to assist smoking cessation. These include the observations that depressive symptoms may be associated with continuation of nicotine use (Kinnunen, Doherty et al. 1996) and that some antidepressant medications may act on specific neural receptors or pathways implicated in nicotine dependence (Stead, Perera et al. 2008). For example, bupropion has antagonist activity at the nicotinic acetylcholine receptor (Slemmer, Martin et al. 2000). Bupropion is an atypical antidepressant medication and the first non-nicotine agent approved for smoking cessation. When used alone, the pooled RR for bupropion is similar to that of NRT at 1.69 (95% CI 1.53 to 1.85) (Hughes, Stead et al. 2007).

One placebo-controlled trial showed that bupropion significantly increased cessation rates compared to nicotine patch (NRT) (Jorenby, Leischow et al. 1999). However, two more studies (open-label) that have performed a direct comparison of bupropion and NRT and failed to show any significant difference; pooled analysis of these studies do not provide strong evidence that either treatment is more effective than the other (Gorecka, Bednarek et al. 2003, Hughes, Stead et al. 2007).

Nortriptyline, a tricyclic antidepressant, has been shown to increase the probability of long term cessation (pooled RR 2.03, 95% CI 1.48 to 2.78) (Hughes 2007). A study that compared the smoking cessation rates achieved with bupropion or nortriptyline did not show a significant difference (Wagena, Knipschild et al. 2005).

Both of these drugs are not risk-free. Allergic reactions to bupropion have been reported at low incidence with 1 to 3 in 1000 in clinical trials (Hughes, Stead et al. 2007). Delayed hypersensitivity has also been reported with bupropion use at very low rates, 0.12% (Ferry and Johnston 2003). However, unlike bupropion, there are a limited number of trials of nortriptyline and only a small number of participants have been studied. In addition to their weak effectiveness (low quit rates), both bupropion and nortriptyline have potential for serious adverse effects. The risk of seizures with bupropion is reported to be approximately 1 in 1000 (Dunner, Zisook et al. 1998). Although unclear, there have been associations made between bupropion use and suicide risk (Stead, Perera et al. 2008). Forty-six cases of suicidal ideation and 29 cases of suicidal behaviour have been reported for bupropion by the FDA (US FDA 2009). Nortriptyline can be lethal in overdoses (Battersby, O'Mahoney et al. 1996).

1.4.3 Clonidine

Clonidine is a second-line therapy drug for smoking cessation. It is currently FDA-approved for the treatment of hypertension (Nides, Oncken et al. 2006). Clonidine is an alpha-2-adrenergic agonist that reduces the sympathetic outflow in the central nervous system (CNS) producing central effects such as sedation and anxiolysis (Gourlay and Benowitz 1995; Nides, Oncken et al. 2006). It is thought that clonidine is effective for smoking cessation due to its anxiolytic ability, as anxiety is a feature of the nicotine craving and withdrawal syndrome.

Six clinical trials were included in a Cochrane review to assess the efficacy of clonidine for smoking cessation. Studies used either oral tablets (dose range 0.15 to 0.45 mg/day) or transdermal patches (doses 0.1 to 0.3 mg/day). Pooled results showed that clonidine nearly doubled the abstinence rate with an odds ratio of 1.89 after 12 or more weeks (Gourlay, Stead et al. 2004).

Clonidine use is associated with a high incidence of dose-dependent adverse reactions. These include sedation, postural hypotension, dry mouth, and constipation (Gourlay, Stead et al. 2004). There were also reports of allergic reactions to the transdermal patches. Clonidine may also increase the effects of drugs that possess depressive effects on the CNS. Caution is needed when clonidine is

co-administered with β-blockers and calcium channel blockers (Nides, Oncken et al. 2006). Abrupt cessation of clonidine can lead to withdrawal symptoms such as agitation, headache, tremor, and rebound hypertension (Nides, Oncken et al. 2006).

1.4.4 Varenicline

Varenicline was approved by the US FDA in 2006 and it is the only nicotine partial agonist currently (mid 2015) licensed for smoking cessation. Varenicline has a higher affinity for the $\alpha_4\beta_2$-nAChR (Ki=0.4 nM) than nicotine (Ki=16.1 nM)(Rollema, Shrikhande et al. 2010) and moderate affinity for the serotonin 5-HT_3 receptor (Boido, Tasso et al.). In a functional electrophysiological assay in *Xenopus* oocytes, it has been shown that varenicline had 68% agonist activity at the human $\alpha_4\beta_2$-nAChR compared to the same concentration of nicotine and simultaneous exposure to varenicline reduced nicotine effects by 34% (Boido, Tasso et al. 2003). The agonistic action of nicotine at the $\alpha_4\beta_2$-nAChR is believed to be an important mechanism underlying nicotine dependence (Tapper, McKinney et al. 2004) and thus identification of selective ligands is considered to be an important area of research for the treatment of nicotine dependence (Coe, Vetelino et al. 2005). A partial agonist for the $\alpha_4\beta_2$-nAChR would theoretically elicit a moderate release of dopamine to relieve nicotine withdrawal and at the same time competitively block nicotine to reduce the rewarding effects of cigarette smoking (Zhu 1996, Tutka and Zatonski 2006). When compared with placebo, varenicline has been shown to increase rates of long-term smoking cessation (pooled RR 2.27, 95% CI 2.02 to 2.55) (Cahill, Stead et al. 2011) and to be more effective than bupropion in achieving abstinence at one year (pooled RR 1.52, 95% CI 1.22 to 1.88) (Cahill, Stead et al. 2011). Varenicline has been reported to be well tolerated, even when used for longer periods than the standard dosing regimen of Champix® (a commercial form of varenicline marketed by Pfizer) (Tonstad, Tonnesen et al. 2006). The most frequent adverse effect reported with the use of varenicline is nausea, which was generally reported to be mild or moderate in most studies (Gonzales, Rennard et al. 2006, Aubin, Bobak et al. 2008). However, post-marketing safety data suggest that varenicline use may be associated increased risk of depression and suicidal ideation (FDA 2008), although a subsequent cohort study found no clear evidence of these associations (Gunnell, Irvine et al. 2009). There have also been reports of an increased risk of cardiovascular side effects with varenicline use in people with cardiovascular disease, but no strong evidence has been found to support this so far (Singh, Loke et al. 2011, Harrison-Woolrych, Maggo et al. 2012, Prochaska and Hilton 2012).

Table 1.1. Pooled relative risks for first and second-line pharmacological therapies for smoking cessation.

Medication	Relative risk over placebo (95% Confidence interval)
Nicotine replacement therapy (Stead, Perera et al. 2008)	1.58 (1.50-1.66)
Bupropion (Hughes, Stead et al. 2007)	1.69 (1.53-1.85)
Nortriptyline (Hughes, Stead et al. 2007)	2.03 (1.48-2.78)
Clonidine (Gourlay, Stead et al. 2004)	1.63 (1.22-2.18)
Varenicline (Cahill, Stead et al. 2012)	2.27 (2.02-2.55)

1.4.5 Electronic cigarettes

Electronic cigarettes (ECs) are battery powered, electronic devices that deliver nicotine as a vapour. There are two published randomised controlled trials that have compared EC with placebo EC (no nicotine). In the ASCEND trial (Bullen, Howe et al. 2013), smokers were assigned to ECs with nicotine, placebo EC (no nicotine) or nicotine (21 mg) patches from 1 week pre-cessation to 12 weeks after quit day, with low intensity behavioural support. At 6 months follow-up, the carbon monoxide-verified continuous abstinence rates were 7.3% for ECs with nicotine, 5.8% for patches and 4.1% for placebo ECs. However, the differences between these groups were not statistically significant. In the ECLAT trial (Caponnetto, Campagna et al. 2013), smokers were randomised to one of three groups. Group A received ECs containing 7.2 mg nicotine cartridges for 12 weeks, Group B received ECs containing 7.2 mg nicotine cartridges for 6 weeks then 5.4 mg nicotine cartridges for an additional 6 weeks and Group C received placebo cartridges (no nicotine) for 12 weeks. At one year follow-up, carbon monoxide-verified abstinence rates were higher in the groups that received nicotine ECs (13% in Group A and 9% in Group B) than the group that received placebo EC (4%). However, when the two nicotine EC groups were compared with the placebo group, the difference was not statistically significant (McRobbie, Bullen et al. 2014). Given the limited number of randomised controlled trials on ECs, more research should be undertaken on the effectiveness and safety of the use of these devices.

1.5 Non-pharmacological interventions

In a review of the effectiveness of behavioural interventions for smoking cessation, it was shown that individual counselling was more effective than no intervention in achieving long term smoking cessation (pooled RR 1.39, 95% CI 1.24 to 1.57) (Hartmann-Boyce, Lancaster et al. 2014). However, there was no evidence to suggest that intensity of counselling was important; the effects of intensive counselling versus brief counselling were found to be similar (pooled RR 0.96, 95% CI 0.74 to 1.25) (Lancaster and Stead 2005). In another pooled analysis, it was shown that providing brief physician advice to smokers resulted in a significant increase in quitting than usual care (no advice) (pooled RR 1.66, 95% CI 1.42 to 1.94) (Stead, Bergson et al. 2008). A study involving 3,030 smokers who were randomised to receive one of: a self-help quit kit, a quit kit plus one telephone counselling or a quit kit plus multiple telephone counselling (up to six times) showed that quit rates were significantly higher in the counselling groups than the self-help group. At 12 months follow-up, quit rates for individuals who made a quit attempt were lowest for the self-help group (5.4%) followed by single counselling (7.5%) and multiple counselling (9.9%) suggesting that the effectiveness of behavioural interventions may be related to the frequency of the support (Zhu, Stretch et al. 1996).

1.6 Cytisine

There has been growing interest in cytisine, an alkaloid found in plants such as Golden Rain (*Cytisus laburnum*), as an aid to smoking cessation because of its current low cost compared to other existing pharmacotherapies (Etter, Lukas et al. 2008, Tutka 2008). Cytisine has a similar structure to nicotine and varenicline and is a partial agonist for the $\alpha_4\beta_2$-nAChR (Rollema, Coe et al. 2007). Cytisine has been used as an aid to smoking cessation since the 1960s in a number of Eastern and Central European countries, but has remained relatively unknown to the rest of the world. Tabex® (commercial form of cytisine marketed by Sopharma) is licenced and approved for use as a smoking cessation medication in a limited number of countries (Tutka 2008).

To date, few human studies have been conducted on cytisine as a smoking cessation medication. Most of these studies were trials that were undertaken before the advent of good clinical practice guidelines (GMP) and have methodological limitations which impact on the interpretation of findings (these studies are discussed in Chapter 2). In addition, prior to the undertaking of this thesis, there were no published human cytisine pharmacokinetic data. Significant gaps, therefore, remain in our knowledge of cytisine, including:

- Absorption and dispositional profile of cytisine in humans;
- Metabolism of cytisine in humans;
- Possibility for drug-drug interactions of cytisine;
- Cytisine's effect on physiological measures such as heart rate, blood pressure and respiratory rate;
- Cytisine's effect on psychological measures such as cigarette cravings/urge to smoke, nicotine withdrawal, mood and smoking satisfaction;
- Concentration-effect relationships for cytisine in human smokers;
- Understanding of the rationale for the current recommended dosing regimen of Tabex®.

1.7 Study rationale

Pharmacokinetic studies are essential in understanding drug disposition. Up to now, trials of cytisine have used the recommended dosing regimen developed by Sopharma. This dosing regimen is complex and may be sub-optimal and may also limit adherence to therapy: no public information exists on the rationale for this dosing regimen. Investigating cytisine pharmacokinetics in humans may allow refinement of the current Tabex® dosing regimen that increases efficacy and clinical effectiveness. Pharmacokinetic data can, therefore, inform the design of future trials that explore different dosing regimens. Furthermore, pharmacokinetic investigations of cytisine can provide data that can be used to investigate concentration-effect relationships in smokers and may be useful in identifying the optimal concentration required to produce the desired effect in smokers.

Currently, there are efforts to get cytisine into clinical use internationally. The drive to move cytisine research forward has also been highlighted in a workshop titled, *Cytisine: A Globally Affordable Treatment for Tobacco Dependence?* (Walker, West et al. 2014) which was held in February 2014 as part of the 2014 Society for Research on Nicotine and Tobacco (SRNT) 20th Annual meeting. In New Zealand, active research has been conducted on cytisine (Walker, Howe et al. 2011, Walker, Howe et al. 2014). Information on the clinical pharmacokinetics of cytisine and more safety data in humans is needed before medicines regulatory authorities (New Zealand Medsafe) can approve the registration of cytisine as a smoking cessation medication in New Zealand.

1.8 Aims of the thesis

The primary aim of this thesis was to obtain basic pharmacokinetic data for cytisine in humans. The objectives were:

1. To develop a validated analytical assay that can be applied to clinical studies of cytisine
2. To describe single-dose pharmacokinetics of cytisine in humans
3. To study the effects of cytisine on physiological (heart rate, blood pressure, respiratory rate) and psychological (cigarette craving, nicotine withdrawal, mood and smoking satisfaction) measures in smokers
4. To measure plasma cytisine concentrations in smokers who follow the standard dosing regimen of Tabex®
5. To explore whether the effects of cytisine can be related to the plasma cytisine concentrations in human smokers

1.9 Outline of thesis

This chapter has introduced the topic and provided a brief overview of the current pharmacological treatments used in smoking cessation. The main objectives of the thesis have been presented.

Chapter 2 summarises the background literature on preclinical and clinical studies of cytisine.

Chapter 3 describes the development of analytical methods for cytisine and the steps taken to validate the assay for the determination of cytisine in human blood and urine samples. A human liver metabolism study of cytisine is also described.

Chapter 4 describes a clinical study that was undertaken to determine the cytisine concentrations in plasma over 24 hours following a single dose (3 mg) and cytisine's effects on physiological measures over this time period.

Chapter 5 describes a subsequent clinical study that measured cytisine's effect on craving, withdrawal, smoking satisfaction, mood and smoking cessation in smokers who were instructed to adhere to the recommended dosing regimen of Tabex®. Plasma concentrations of cytisine during the dosing period were measured to explore whether these effects measured could be related to the plasma concentrations of cytisine in smokers during the recommended dosing period. Safety data are also presented.

Finally, Chapter 6 summarises the main findings in this thesis and presents a general discussion. Future research directions are outlined.

1.10 Summary

A small number of smokers quit successfully without the use of any aids to smoking cessation. A number of pharmacological treatments exist to aid smoking cessation including NRT products, bupropion and varenicline. Cytisine, a plant alkaloid that is a structural analogue of varenicline, is not yet approved as a smoking cessation medication in many countries outside of Central and Eastern Europe. Like varenicline, cytisine has a similar structure to nicotine and is a partial agonist for the $\alpha_4\beta_2$-nAChR. There is growing interest in cytisine because of its potential to be a much more affordable treatment for smoking cessation than any other currently available pharmacotherapy on the market. However, there are significant gaps in the knowledge of cytisine that are required to be filled.

The next chapter will present a background literature review of the preclinical and clinical studies of cytisine.

Chapter 2 Background literature review

2.1 Introduction

Cytisine has been commercially available for over 50 years as a smoking cessation medication in several countries (largely in parts of Eastern and Central Europe). Cytisine is marketed by a Bulgarian pharmaceutical company called Sopharma under its trade name Tabex®. Recently, cytisine has received interest from researchers due to the success of its synthetic analogue varenicline, which is approved by the FDA as a smoking cessation medication. The current key advantage of cytisine in smoking cessation is its current low cost (Etter, Lukas et al. 2008): the current cost of cytisine treatment is under $NZ 40 for 25 days which is approximately less than 10% of the unsubsidised cost of nicotine replacement therapy or varenicline treatment (Walker, Howe et al. 2014).

Another appeal of cytisine as a smoking cessation agent is that it is marketed as “herbal”, “natural” or “non-medical” (www.tabex.net); this could be an advantage in attracting certain smoking populations (even though nicotine is also a plant-derived compound).

Cytisine is a plant alkaloid that is structurally related to nicotine and like varenicline, and is a partial agonist for nicotinic receptors (Rollema, Coe et al. 2007); in particular it shows a high affinity for the $\alpha_4\beta_2$ subtype which is thought to be the main receptor that mediates central effects of nicotine (Coe, Brooks et al. 2005). Thus cytisine is thought to act in the same way as varenicline, i.e. to aid smoking cessation by reducing the severity of tobacco withdrawal symptoms by moderately stimulating the release of dopamine in a way that does lead to dependence and by reducing the rewarding effects of smoking by its antagonistic actions (Tutka and Zatonski 2006).

Despite over 50 years of use as a smoking cessation medication, cytisine is not listed in FDA recommendations or guidelines for such purposes and until recently, there have been few publications in English about the use and effectiveness of the drug. Furthermore, most human studies were early trials that were undertaken before the introduction of good clinical practice guidelines (GMP) (Paun and Franze 1968, Benndorf, Scharfenberg et al. 1969, Benndorf, Scharfenberg et al. 1970, Scharfenberg, Benndorf et al. 1971, Schmidt 1974, Marakulin, Komarov et al. 1984) and fail to meet modern regulatory standards and requirements (Etter 2006). These studies are discussed later in the chapter.

2.2 Preclinical pharmacology of cytisine

2.2.1 Pharmacodynamics

Cytisine binds predominantly to the $\alpha_4\beta_2$-nAChR with nanomolar affinity (Ki=0.124 – 2.4 nM) (Pabreza, Dhawan et al. 1991, Boido, Tasso et al. 2003). Experiments with *Xenopus* oocytes or cell cultures show that the affinity of cytisine for nAChRs is the highest for $\alpha_4\beta_2$ subtype followed by $\alpha_4\beta_4$, $\alpha_2\beta_4$, $\alpha_3\beta_2$, $\alpha_3\beta_4$ and α_7 (Parker, Beck et al. 1998, Coe, Brooks et al. 2005). Experiments that compared the binding of cytisine and nicotine to $\alpha_4\beta_2$-nAChR showed that cytisine has approximately 7-fold greater affinity for the receptor than nicotine (Imming, Klaperski et al. 2001, Coe, Brooks et al. 2005). In an experiment with rat prefrontal cortical slices, cytisine increased the release of [^{3}H]-dopamine to a similar extent as nicotine and increased the release of [^{3}H]-norepinephrine more than nicotine (Rao, Correa et al. 1996). Cytisine was not shown to affect [^{3}H]-serotonin release (Rao, Correa et al. 1996). In a study that compared the current evoked using 10 µM cytisine to 10 µM nicotine, it was estimated that cytisine's agonistic activity was only 56% of that of seen with nicotine (Coe, Vetelino et al. 2005). Another study showed that cytisine increased the maximum release of [^{3}H]-dopamine in rat striatal slices that was approximately half of that observed with nicotine (Abin-Carriquiry, Voutilainen et al. 2006). An in-vivo study in rats also showed that cytisine administration increased the dopamine turnover which was only approximately 40% of that seen with nicotine administration and reduced nicotine effects by 36% in animals treated concurrently with cytisine and nicotine (Coe, Brooks et al. 2005).

Based on these findings, it is hypothesised that cytisine has a dual action (partial agonist) implicated in smoking cessation. Cytisine binds to $\alpha_4\beta_2$-nAChR with high affinity and moderately increases dopamine release in the mesolimbic system (agonist actions): this would relieve some withdrawal symptoms in abstinent smokers (Rollema, Coe et al. 2007). At the same time, cytisine blocks the effects of nicotine (antagonistic action) (i.e. reduces the reward from smoking). Cytisine may have a lower potential for addiction because of its lower efficacy compared to nicotine in eliciting dopamine release.

2.2.2 Pharmacokinetics

The absorption, distribution, metabolism and excretion of cytisine has not been characterised in humans and the information available on the preclinical metabolism and pharmacokinetics is scarce and therefore the information presented here pertains to animal studies.

2.2.2.1 *Absorption*

A study in mice found that following intravenous and oral administrations of 2 mg/kg cytisine, the bioavailability was approximately 42% and the time to reach maximum blood concentration (T_{max}) was 120 minutes (Klocking, Richter et al. 1980). In rabbits, 5 mg/kg cytisine was administered and the oral bioavailability was 34% and T_{max} was observed after 35 minutes (Tutka and Zatonski 2006). Astroug et al. investigated the pharmacokinetics of cytisine in rabbits after oral (5 mg/kg) or intravenous (1 mg/kg) administration and found the bioavailability to be 32.18% (Astroug, Simeonova et al. 2010). T_{max} was observed within 35 minutes on average in rabbits following a 5 mg/ kg oral dose (Astroug, Simeonova et al. 2010).

A transdermal therapeutic system was also studied in rabbits which resulted in two steady states of which the first phase lasted for 24 hours and had double the blood concentration as the second phase which lasted for 3 days thereafter (Sariev, Zherdev et al. 1999).

2.2.2.2 *Distribution*

Following a single intravenous 2 mg/kg dose in mice, the highest cytisine concentration was reported in the liver, adrenal glands and kidneys at 30 minutes post-dose (Klocking, Richter et al. 1980). However, this study only measured the radioactivity (^{3}H-cytisine) in tissues and body fluids and did not report cytisine plasma concentration. Cytisine was also detected in the bile with a concentration that was 200 times greater than that of the blood 3 to 4 hours following an intravenous administration.

After administration of 1 mg/kg cytisine in rats, the concentration of cytisine in the brain was found to be 145 ng/mL, which was only approximately 28% that of the plasma concentration (516 ng/mL) at 15 minutes post-dose (Reavill, Walther et al. 1990). This finding suggested that cytisine is a weak penetrator of the blood-brain barrier compared to nicotine.

2.2.2.3 *Metabolism and elimination*

In mice, 90 to 95% of a cytisine dose is excreted unchanged in the urine (Klocking, Richter et al. 1980). In rabbits, clearance following an oral (5 mg/kg) and intravenous (1 mg/kg) administration were 167 mL/min and 43 mL/min respectively (Tabex , Sopharma 2008). Approximately 11% and 3%

of cytisine was excreted in the faeces of rabbits unchanged after oral and intravenous administration respectively (Astroug, Simeonova et al. 2010).

The half-life observed following a single intravenous dose of cytisine was 200 minutes in mice (Klocking, Richter et al. 1980). The elimination half-life in rabbits was 52 minutes and 37 minutes following an oral and intravenous administration respectively (Sopharma 2008). In rabbits, half-life of cytisine was 51 minutes following an oral administration and 36 minutes following an intravenous administration (Astroug, Simeonova et al. 2010). In mice, the recovery was approximately 32% of the dose in urine over 24 hours after intravenous administration and only 18% of the dose was recovered in urine over 24 hours following oral administration of the drug (Klocking, Richter et al. 1980).

A toxicological case study reported that there are unpublished results from an experiment that screened urine from rats after cytisine administration (Musshoff and Madea 2009). This study did not detect any metabolites of cytisine.

2.2.3 Animal toxicity

The toxicity of cytisine has been studied in rodents, dogs, cats and horses (Angelova 1971, Tutka and Zatonski 2006). Following acute exposure to cytisine, the lethal dose, 50% (LD_{50}) values in mice appeared to be dependent on route of administration and to some extent by sex (Angelova 1971). In male mice the LD_{50} values were 2.3 mg/kg intravenous, 13 mg/kg subcutaneous, 13 mg/kg orally, whereas the female mice had higher LD_{50} values with 3.1 mg/kg intravenous, 13 mg/kg subcutaneous, 29 mg/kg orally (Paskov and Dobrec 1953). In rats, the LD_{50} value was 9 mg/kg intraperitoneal, 11 mg/kg subcutaneous and 38 mg/kg orally. The most common symptoms were related to toxicity of the gastrointestinal tract such vomiting (Tutka and Zatonski 2006).

2.3 Clinical studies of cytisine

2.3.1 Literature search methods

Studies were identified using several methods including:

- An initial search on Databases including Google scholar, Pubmed (includes Medline) and Embase using terms: “cytisine” or “Tabex”
- The reference list of identified papers
- Personal communication with authors of cytisine papers (Dr Natalie Walker (Walker, Howe et al. 2014) and Professor Piotr Tutka (Tutka 2008))
- Weekly Google Scholar alerts for term “cytisine”

2.3.2 Overview of studies

As mentioned earlier, early trials of cytisine as a smoking cessation therapy published in the 1960s and 1970s have several design shortcomings (e.g. uncontrolled, non-randomised). These studies lack detailed documentation of important information such clear definitions of the outcome measure (abstinence criteria) and most of these older studies did not use biochemical verification of abstinence. In addition, most of these studies lacked a long-term follow-up that is widely accepted for smoking cessation trials as a standard (> 6 months follow-up (West, Hajek et al. 2005)). Most of these trials have used the standard dosing regimen of Tabex® (Table 2.1) which was developed in the 1960s, but does not appear to be evidence-based.

Although observational studies do not provide a strong evidence of a relationship between cytisine and abstinence, these studies do suggest that cytisine is safe at the doses studied and that it may have some effect in smoking cessation. More recently, well-designed randomised, double-blind, controlled trials have shown that cytisine is more effective in achieving continuous abstinence than placebo.

Table 2.1. Tabex® (1.5 mg cytisine) dosing regimen.

Days	Dosing regimen	Dose per day (mg)
1 – 3	1 tablet every 2 hours (maximum of 6 tablets)	9
4 – 12	1 tablet every 2.5 hours (maximum of 5 tablets)	7.5
13 – 16	1 tablet every 4 hours (maximum of 4 tablets)	6
17 – 20	1 tablet every 5 hours (maximum of 3 tablets)	4.5
21 – 25	1 tablet every 6 hours (maximum of 2 tablets)	3

Source: www.tabex.net

2.3.3 Smoking cessation trials

An uncontrolled trial (Granatowicz 1976) involved 1968 smokers, some of whom received behavioural support or received other drugs, who took Tabex® for 25 days. At 6 months follow-up, 70% of participants who took cytisine were reported to be abstinent. The number of participants at follow-up is unknown. An observational uncontrolled study (Kempe 1967) involved 30 male smokers who were given Tabex® for 17 days. At 1 month follow-up, approximately 63% of the participants were abstinent. In an observational study involving 87 smokers, cytisine was reported to be effective in helping smokers to quit but no follow-up period was reported (Stoyanov and Yanachkova 1965). An uncontrolled, open-label trial was conducted in Poland involving 436 smokers who adhered to the standard dosing regimen of Tabex (25 days with a target quit day on day 5) (Zatonski, Cedzynska et al. 2006). Participants received minimal behavioural support and the main outcome measure was self-reported continuous abstinence (defined as no cigarettes at all), verified using expired carbon monoxide (CO) (<10 ppm) for 12 months. At 12 months, 13.8% of the participants achieved abstinence. Dry mouth was reported in 35% of the participants, whilst nausea and gastric disturbances were reported in 10% of the participants. Rate of discontinuation of cytisine due to adverse effects was 15.5%. No serious adverse events were reported.

Placebo-controlled trials of cytisine show that cytisine increases the chance of quitting compared with placebo (Table 2.2). In a non-randomised trial, 366 participants received cytisine and 239 participants received placebo for 17 days. It is unclear whether any form of behavioural support was given to participants (Paun and Franze 1968). At 8 weeks, 55% of the participants who received cytisine had quit smoking compared with 33% in the placebo group. However, there was no validation of abstinence.

In a double-blind placebo-controlled randomised trial involving 1214 smokers (Scharfenberg, Benndorf et al. 1971), half of the participants took cytisine and the other half took placebo for 20 days following this regimen: 6 tablets/day for the first three days, 5 tablets/day on days 4 – 12, 4 tablets/day on days 13 – 16 and 3 tablets/day on days 17 – 20. No behavioural support was given to participants. Outcome measures were self-reported abstinence (undefined) at 4 weeks, 6 months and 2 years. For all follow-up time points, cytisine was significantly more effective than placebo. At 2 years, the abstinence rate in the cytisine group and placebo group were 21% and 13% respectively. The risk of bias, however, is uncertain with this study as no information is provided on how the randomisation was undertaken (selection bias), outcome data reported are incomplete (attrition bias) and not all expected and predicted outcomes were reported (reporting bias). In addition, the treatment duration is shorter than the recommended dosing regimen and outcomes measures were not clearly defined (other than abstinence) and did not use biochemical validation. At 2 years, the response rate (via mail) was only 66%.

In another study, 2475 smokers received one of 16 smoking cessation preparations which included Tabex (n=250) and placebo (n=270) (Schmidt 1974). This study found that cytisine was significantly more effective in achieving abstinence over placebo at follow-up at 4 weeks. At 3 months, no significant differences were detected between cytisine and placebo. Abstinence was not biochemically validated.

Although not placebo-controlled, a study conducted by Marakulin and colleagues compared smokers who received autogenic training (a form of relaxation therapy) (n=232) or autogenic training plus cytisine treatment for 21 days (n=388) (Marakulin, Komarov et al. 1984). At the 3 week follow-up, the quit rates reported in this study was higher in the autogenic training plus cytisine group (70.1%) than the autogenic training group (53%). Abstinence was not validated and it is not clear whether participants were randomised to each group.

There is also an unpublished randomised placebo-controlled trial that has been described in a systematic review of cytisine (Hajek, McRobbie et al. 2013). At the 26 days follow-up, the abstinence rate in the cytisine group was higher than the placebo group.

Although the studies described above were controlled, there are several design limitations such as lacking a clear definition of abstinence criteria and whether individuals were randomly allocated to the treatment groups. The risk of bias for these studies is unclear.

To date, there are only two double-blind placebo-controlled parallel-group randomised controlled trials of cytisine that conform to the modern regulatory standards (Vinnikov, Brimkulov et al. 2008, West, Zatonski et al. 2011). The Vinnikov study involved 171 adult smokers working in the mining industry who wanted to quit smoking (Vinnikov, Brimkulov et al. 2008). Mean FTND score was 5.3 at baseline and 86% of the participants had tried to quit smoking previously. Participants were randomised into 2 groups, either to receive Tabex® (n=85) or matching placebo (n=86) for 25 days following this regimen: 6 tablets/day on days 1-3, 5 tablets/day on days 4-12, 4 tablets/day on days 13-16, 3 tablets/day on days 17-20, 2 tablets/day on days 21-22 and 1 tablet/day on days 23-25. Participants were asked to reduce smoking by half during the first 3 days and target quit day was on day 5. Participants also received behavioural counselling but no information was reported on the frequency or type of counselling. Baseline characteristics of participants were very similar for both groups (age and smoking status). The primary outcome in the study was CO-validated (8 ppm or less) continuous abstinence rate (defined as no cigarettes at all) from day 5 to week 8. At week 8, the continuous abstinence rate was greater in the cytisine group compared with placebo group (10.6% vs 5.7%) but this was not statistically significant. The secondary outcome was CO-validated continuous abstinence rate from day 5 to week 26. At 26 weeks, the abstinent rate in the cytisine group (10.6%) was significantly greater than placebo group (1.2%) (RR 8.73 95% CI: 1.13 to 67.61) (Vinnikov, Brimkulov et al. 2008). It is noteworthy that the majority of the participants were males (only 1 female in the cytisine group and 4 females in the placebo group), thus these results may not be representative of the female population of smokers. Rates of discontinuation of cytisine use due to adverse events were the same in both cytisine and placebo groups (4.7%). The most common adverse effects reported in the study were dyspepsia, nausea and headache, all of which occurred at similar rates in both groups (reported by one or two participants in each group).

The West study was a single-centre study involving 740 healthy adult smokers (West, Zatonski et al. 2011). The participants were highly dependent smokers (mean FTND score 6.2) and 82% of the participants had made a previous quit attempt. Individuals were randomised to cytisine (Tabex®) or matching placebo for 25 days (370 per group). The dosing regimen was as follows: 6 tablets/day for the first three days, five tablets/day for days 4 – 12, 4 tablets/day for days 13 – 16, 3 tablets/day for days 17 – 20 and 2 tablets/day for days 21 – 25. The target quit day was on day 5. Participants were provided with quitting advice at the first visit but the authors report that behavioural support was minimal throughout the rest of the study. However, participants received phone calls on the target quit day and 1 week later. The primary outcome was abstinence at 12 months after end of treatment, defined as smoking fewer than 5 cigarettes during 6 months after end of treatment and none in the

week prior to visit, as confirmed with biochemical verification (expired CO less than 10 ppm). At the end of the study period, the rate of sustained 12-month abstinence was 8.4% in the cytisine group and 2.4% in the placebo group (RR 3.44 95% CI: 1.66 to 7.13) (West, Zatonski et al. 2011). Secondary outcomes were sustained CO-verified abstinence at 6 months, 2 week PPA at 4 weeks, 7-day point prevalence for abstinence (PPA) at 12 months. Adverse events and serious adverse events were also reported. Gastrointestinal adverse events (dry mouth, dyspepsia, nausea, stomach-ache, etc.) were more frequently reported in the cytisine group (51%) than placebo group (30%) although drug discontinuation rates in both groups were similar.

2.3.3.1 *Pooled analysis of randomised, controlled trials*

Of the studies that investigated the effectiveness of cytisine as an aid to smoking cessation, the Vinnikov study and the West study (Vinnikov, Brimkulov et al. 2008, West, Zatonski et al. 2011) have a low risk of bias and deemed appropriate for pooled analysis (Cahill, Stead et al. 2012). Both studies have avoided risk of selection bias by using an independent statistician for randomisation. Risk of reporting bias was also low for the two studies as both studies reported all expected and predicted outcomes. A pooled analysis has been done with the results of these two studies of cytisine in a Cochrane Review (Cahill, Stead et al. 2011). These two trials of cytisine (Vinnikov, Brimkulov et al. 2008; West, Zatonski et al. 2011), with a total of 937 people studied (470 received cytisine), showed that smokers who used cytisine had a higher chance of achieving abstinence compared with placebo (pooled RR 3.98, 95% CI: 2.01 to 7.87) (Cahill, Stead et al. 2012).

Based on results from these studies, cytisine is a more effective treatment in aiding smoking cessation than placebo, even for smokers who work in a stressful environment (workers in the mining industry). However, absolute abstinence rates reported for the cytisine treated groups are low and reported to be less than 11% (Vinnikov, Brimkulov et al. 2008; West, Zatonski et al. 2011).

Table 2.2. Placebo-controlled studies of cytisine (Tabex®)

First author, Publication year	N Cytisine group	N Placebo group	Treatment duration	Other support	Longest Follow-up period	Quit rate (%) Cytisine group	Quit rate (%) Placebo group	Odds ratio (95% CI), p value
Paun, 1968 (Paun and Franze 1968)	36	270	17 days	Group sessions	8 weeks	41.7	33.5	1.42 (0.65-3.06) 0.34
Benndorf, 1969 [a] (Benndorf, Scharfenberg et al. 1969)	607	607	20 days	None	4-6 weeks	65.1	40.5	2.73 (2.15-3.47) <0.001
Scharfenberg, 1971[a] (Scharfenberg, Benndorf et al. 1971)	607	607	20 days	None	2 years	20.9	13.0	1.77 (1.29-2.43) <0.001
Schmidt, 1974[a] (Schmidt 1974)	250	270	3 weeks	Single information lecture	3 months	27.2	21.1	1.40 (0.91-2.13) 0.10
Vinnikov, 2008[a,b,c] (Vinnikov, Brimkulov et al. 2008)	85	86	25 days	Brochures, counselling	26 weeks	10.6	1.2	10.07 (1.26-217.07)
West, 2011[a,b,c] (West, Zatonski et al. 2011)	370	370	25 days	"minimal amount of counselling"	12 months	8.4	2.4	3.67 (1.65-8.42) <0.001

Notes:
[a] indicate studies that have randomised participants to treatment groups
[b] indicate studies that have used biochemical verification of abstinence
[c] indicate studies that have used Russell Standard outcome (West, Hajek et al. 2005) assessment for abstinence

2.3.4 Cytisine versus nicotine replacement therapy

An open-label, non-inferiority trial was conducted to investigate whether cytisine was at least as effective as nicotine replacement therapy (Walker, Howe et al. 2014). This study involved 1310 daily smokers in New Zealand over the age of 18 who wanted to give up smoking. Smokers were recruited through the national Quitline (New Zealand Quitline). The exclusion criteria for the study were pregnant or breast-feeding, self-reported phaeochromocytoma, systolic and diastolic blood pressure higher than 150 and 100 mm Hg respectively, diagnosis of schizophrenia, self-reported cardiovascular event in the 2 weeks before study or taking other smoking cessation medications. Smokers were randomised to receive cytisine for 25 days (adhering to manufacturer's dosing regimen-see Table 2.1) or nicotine replacement therapy for 8 weeks (n=655 per group). Participants allocated to cytisine received their medication via mail free of charge whilst participants in the nicotine replacement therapy group received vouchers by mail which could be used to purchase nicotine patches (7, 14 or 21 mg) along with gum (2 or 4 mg) or lozenges (1 or 2 mg) or both gum and lozenges from community pharmacies for a small cost (NZ$3). The target quit day was day 5 of the treatment. Low-intensity telephone behavioural support was provided to participants via Quitline. The primary outcome was self-reported continuous abstinence at 1 month (defined as smoking five cigarettes or less since quit day (West, Hajek et al. 2005)). Continuous abstinence rates reported at 1 month was significantly higher in the cytisine group than nicotine replacement therapy (40% and 31% respectively, 95% CI 4.2 to 14.5). Continuous abstinence rate at 2 and 6 months (secondary outcomes) were consistent to the primary outcome. In addition, the median time to relapse was significantly longer in the cytisine group than for nicotine replacement therapy group (52 days vs 11 days). The study found that a higher quit rate was observed in women than men in the cytisine group. Self-reported adverse events were more frequently reported in the cytisine group than in the nicotine replacement group (31% vs 20%), but the occurrence of serious adverse events in both groups were similar (7% vs 6%). Most adverse events were classified as mild or moderate. The most common adverse events reported in the cytisine group were nausea/vomiting and sleep disorders.

This study had low risk of selection bias because participants were randomly allocated using computer software, but as this was an open-label trial, participants and researchers were aware of treatment allocation and thus there may have been reporting bias in favour of cytisine. In addition, self-reported abstinence was not biochemically verified. Nevertheless, the researchers discuss that if this study is similar to other New Zealand Quitline trials which have shown that 79% of those who report abstinence are biochemically confirmed, the continuous abstinence rate in the cytisine group

would still be significantly higher than that seen in the nicotine replacement therapy group. However, participants recruited via Quitline may be more motivated to quit and may not be representative of all smokers in the general population.

No other head-to-head comparison trials between cytisine and other smoking cessation medications have been published.

2.3.5 Reduction of withdrawal and urge to smoke

The CASCAID trial (Walker, Howe et al. 2014) also compared the severity of nicotine withdrawal and urge to smoke between participants who received cytisine to those who received nicotine replacement therapy. Greater reduction in nicotine withdrawal severity, as determined using the Mood and Physical Symptoms Scale (MPSS) (West and Hajek 2004) was observed in participants in the cytisine group (mean ± SD, 2.48 ± 0.20) than the nicotine replacement therapy group (1.72 ± 0.24). This difference was statistically significant (p=0.016) (Walker, Howe et al. 2014). Reduction in the mean urge score has also been shown to be significantly greater in the cytisine group (4.36 ± 0.13) than NRT group (3.80 ± 0.15, p=0.005) (Walker, Howe et al. 2014).

In the same study, smoking satisfaction, measured using the modified cigarette evaluation questionnaire (mCEQ)(Cappelleri, Bushmakin et al. 2007) was decreased in participants who continued to smoke while taking cytisine. Participants in the cytisine group also had a greater reduction in psychological reward and enjoyment of the respiratory tract sensations at 1 month post quit than participants in the NRT group (Walker, Howe et al. 2014).

2.3.6 Studies with other forms of cytisine

A trial involving 62 smokers was conducted in which participants were allocated (it is unclear whether they were randomised) to take buccal film of either cytisine (1.5 mg)(n=23), anabasine (a compound that has a similar structure to nicotine) (0.75 mg)(n=23) or both cytisine (0.75 mg) and anabasine (0.75 mg) (n=15)for 15 days (Ostrovskaia 1994). This study included smokers with underlying diseases such as coronary heart disease, hypertension or diabetes mellitus. At 15 days, 47% of the participants were reported to be abstinent, but results by group were not reported. The authors reported that film containing cytisine (cytisine alone or cytisine plus anabasine) was more effective than anabasine alone. No adverse events were reported. In another study (uncontrolled),

cytisine film patches (described as bio-soluble film on a paper or fabric patches) were compared to anabasine or combination of cytisine and anabasine in 281 smokers (Metelitsa 1987). At 6 month follow-up, the authors reported that the abstinence rate was 50%, but the results by group were not reported.

2.3.7 Qualitative studies

A study interviewed 18 Māori smokers (16 years and over) living in New Zealand to explore whether cytisine would be an attractive option for smoking cessation compared to other cessation products (Thompson-Evans, Glover et al. 2011). The study included both female and male participants who all wanted to give up smoking. Participants were provided with information on cytisine such as the molecular makeup and in which countries cytisine is currently approved. Tabex® tablets or a photo of the tablets were shown to the participants. Participants were also told that cytisine is found in the Kowhai tree (an indigenous tree in New Zealand), although it can also be synthetically produced. Participants were then asked to comment on whether they would be willing to use cytisine if it was available. The study reported that participants in this study would use cytisine and that it may be more "effective" and "acceptable" than other existing smoking cessation medications because cytisine can be extracted from the Kowhai tree (*Sophora tetraptera*), which is a plant that has been used in traditional Māori healing (Rhys 2009). The participants associated the Kowhai tree with Māori traditional healing practices and therefore believed that cytisine might be more appealing to Māori smokers than other smoking cessation products currently available on the market.

2.3.8 Safety and tolerability

In general, rates of discontinuation of cytisine use due to adverse events were similar amongst smokers treated with cytisine or placebo (Vinnikov, Brimkulov et al. 2008, West, Zatonski et al. 2011).

One trial (Walker, Howe et al. 2014) reported that two participants in the study had taken the 100 cytisine tablet course within the first week of the study. No adverse events were reported in these participants. Of the 655 participants, 36 stopped taking cytisine due to an adverse event. The rate of discontinuation in the nicotine replacement group was not reported but the cytisine group had a higher incidence of adverse events than nicotine replacement group (31% and 20% respectively) (Walker, Howe et al. 2014).

2.3.8.1 *Common adverse events*

The most common adverse events associated with cytisine treatment across studies are gastrointestinal disturbances. The Vinnikov trial reported the most common adverse effects as dyspepsia (1.2%), nausea (2.4%) and headache (1.2%), with no significant differences between cytisine and placebo groups (Vinnikov, Brimkulov et al. 2008). The West study reported that 13.8% of the participants in the cytisine group experienced a gastrointestinal symptom(s) such as stomach ache (3.8%), nausea (3.8%), dyspepsia (2.4%) and dry mouth (2.2%) (West, Zatonski et al. 2011). In the same study, 2.2% of the participants in the cytisine group reported dizziness and 1.9% reported headache. The most common adverse events in the cytisine group in the Walker trial were nausea and vomiting (4.6%) and sleep disorders (4.3%) (Walker, Howe et al. 2014).

2.3.8.2 *Intentional overdose*

A case study described a 48 year old woman who was hospitalised after ingesting 40 – 50 cytisine tablets (Stoyanov and Yanachkova 1972). She experienced vomiting, lost consciousness and experienced clonic seizures. After regaining consciousness, she experienced muscle spasms, headache, dizziness, weakness, double vision, dysarthic speech and hypotension. She was released 5 days after and showed no further symptoms. This person took a second overdose of 90 cytisine tablets with scopolamine and hyoscyamine (amounts unknown) and lost consciousness for a short period of time before waking up. She was hospitalised for 7 days. No seizures were reported on this occasion. The person had no liver or other internal organ damage.

2.3.9 Medication adherence

A standard course of cytisine treatment contains a total of 100 tablets which are taken over 25 days, although not every study uses this dosing regimen. In one study, the participants took cytisine for 17 days (Paun and Franze 1968). The dosing regimen used in this study was 1 tablet six times daily from days 1 – 3, 1 tablet five times daily for days 4 – 8, 1 tablet four times daily from days 9 – 13 and 1 tablet three times daily for days 14 – 17. For smokers over the age of 50 and those who were diagnosed with arteriosclerosis, the dosing regimen was 1 tablet six times for the 1st day, 1 tablet five times for the 2nd day, 1 tablet four times for the 3rd day and 1 tablet three times daily for days 4 – 17. At the end of the treatment, 18% of the abstinent participants took more than 50 tablets. Sixty six percent of the abstinent participants took 21 to 50 tablets and 16% took less than 20 tablets. In

another study (Scharfenberg, Benndorf et al. 1971), the dosing regimen used was six tablet for days 1 – 3, five tablets for days 4 – 12, 4 tablets for days 13 – 16 and 3 tablets for days 17 – 20. Half of the participants who quit smoking in another study took fewer than 75 of the 100 tablets. In another study (Walker, Howe et al. 2014), participants reported taking a mean ± SD of 49 ± 24 tablets one week after day 5 when they should have taken 63 tablets. At 1 month following the quit day, participants reported taking a mean ± SD of 72 ± 34 tablets (100 tablets should have been taken). Approximately half of the participants in this study took 80 or more tablets.

In the study by Walker and colleagues (Walker, Howe et al. 2014), the dosing regimen and having to remember to take Tabex® were in the top three reasons why participants in the cytisine group disliked the tablets.

2.4 Questions arising from the literature review on cytisine

A review of the literature has shown that although cytisine is more effective for smoking cessation than placebo, its use is associated with relatively low quit rates (less than 11%) (Vinnikov, Brimkulov et al. 2008, West, Zatonski et al. 2011). If smoking cessation is driven by a pharmacological component, this then leads to a question: can pharmacokinetic information be used to improve the clinical effectiveness of cytisine for smoking cessation? To answer this question, it was necessary to conduct studies that investigated the pharmacokinetics of cytisine in humans – data, which at the start of this PhD were not available. Furthermore, in order to explore the concentration-effect relationships of cytisine in smokers, it was necessary to investigate cytisine's effect on psychological measures important in smoking cessation.

2.5 Summary

Cytisine is a partial agonist for the $\alpha_4\beta_2$-nAChR. By partially activating these receptors, cytisine may reduce the severity of nicotine withdrawal symptoms, thus making quitting a less unpleasant experience, and at the same time reduce the rewarding effects of smoking by blocking the binding of nicotine if a smoker relapses.

There is a paucity of well-designed controlled clinical trials with cytisine. Observational studies show that cytisine may have some effect in aiding smokers to quit. There are several placebo-controlled

trials of cytisine, but only two of these trials conform to the modern regulatory standards. These trials have shown that cytisine is relatively safe and more effective than placebo in achieving long term continuous abstinence. However, the absolute quit rates reported in the cytisine group for both studies were low. Cytisine may also be more effective than nicotine replacement therapy in achieving long term abstinence in smokers motivated to quit smoking. However, there is no published rationale for the standard dosing regimen of Tabex® and thus it is unclear whether the cytisine doses studied are optimal doses for smoking cessation.

The next chapter will describe the development and validation of an analytical assay for cytisine that will be used to support the pharmacokinetic studies described in this thesis.

Chapter 3 Development and validation of HPLC-UV and LC-MS methods for the detection and quantification of cytisine in commercial forms of cytisine and human plasma and urine

3.1 Introduction and overview

Cytisine, a plant alkaloid, is the active substance of Tabex®, a smoking cessation medication that has been marketed in Eastern Europe since the 1960s (Stoyanov and Yanachkova 1965, Stoyanov 1967). Interest in getting cytisine into the clinic is growing in the hope that it will be an inexpensive option than other pharmacotherapies currently available for smoking cessation (Aveyard and West 2013, Hajek, McRobbie et al. 2013, Samet 2014).

Despite more than five decades of use, there remains very limited knowledge on the pharmacology of cytisine. Animal data exist (Lee, Siriarayapon et al. 2004, Astroug, Simeonova et al. 2010), but no publicly available information was found for the pharmacokinetics/metabolism of cytisine in humans. In order to study the clinical pharmacology of cytisine, an analytical assay must first be developed and validated in appropriate biological matrices (human blood, plasma or urine).

An analytical method for the determination of cytisine in rabbit serum has been described (Astroug, Simeonova et al. 2010). This method used high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection. Cytisine was administered orally and intravenously with doses of 5 mg/kg and 1 mg/kg respectively. However, the standard dosing regimen of Tabex® recommends that a person takes 1 tablet (1.5 mg cytisine) every 2 hours (maximum of 6 tablets per day) as a starting dose. The frequency of dosing together with the timing between doses change throughout the course of the treatment (25 day course), eventually the last dosing regimen changes to 1 tablet every 6 hours (maximum of 2 tablets per day) (Sopharma 2008). The large discrepancy between dosages studied in animals and the recommended human dose suggests that a more sensitive method is required for the study of the pharmacokinetics of cytisine in humans following recommended dosing regimen. Several other methods for the determination of cytisine have been reported in the literature including methods that have not been validated in human tissues (Ding, Yu et al. 2005, Astroug, Simeonova et al. 2010, Wang, Guo et al. 2012) as well as methods that have been shown to be applicable for herbal intoxication or drug abuse cases (Beyer, Peters et al. 2007, Pietsch, Gunther et al. 2008, Ng, Ching et al. 2013). However, human pharmacokinetic analysis has

not been conducted using these methods and no methods have yet studied the commercial forms of cytisine.

To date, there are two types of commercial products that contain cytisine that are marketed as an aid to smoking cessation. One is the oral tablet (Tabex®) and the other is an oral buccal strip. The oral buccal strip has been marketed in Australia and the Philippines (Quit4Good Nicotine Free Oral Strips and QNE®). Each strip is reported to contain 1 mg of cytisine (instead of 1.5 mg as in tablets), but uses the same recommended dosing regimen as Tabex®.

Two validated analytical assays of cytisine are described in this chapter. Firstly, an HPLC-UV assay was developed and validated for analysis of the two commercial products of cytisine: Tabex® tablets and Quit 4 Good Nicotine Free Oral Strips. Plasma and urine samples taken from one non-smoking, healthy volunteer, who had taken Tabex® were then analysed to investigate whether this method was sensitive enough to detect levels of cytisine following oral administration. Although the usual starting dose is 1.5 mg, a higher dose (3 mg) was selected to increase the chances of detecting cytisine in human tissue. For simplification, a single dose was chosen over the recommended split dosing. However, the sensitivity of the HPLC-UV method was insufficient (not fit for purpose) to detect cytisine in human plasma after the administered dose. Therefore, it was necessary to develop and validate a more sensitive method using high performance liquid chromatography coupled with mass spectrometry (LC-MS) that could be used to investigate pharmacokinetics of cytisine in subsequent human studies.

Investigating drug metabolic pathways can help identify major routes of drug elimination, sources of between-subject variability and potential drug-drug interactions (DDIs). It can also identify possible active metabolites which may produce toxicity or lead to sub-therapeutic drug concentrations. Thus drug metabolism studies are a key component of any drug evaluation.

No published studies have investigated whether cytisine is a substrate for any hepatic enzymes. Animal studies have shown elimination of cytisine is not via metabolism since cytisine is excreted renally as an unchanged drug (>90%) (Klocking, Richter et al. 1980, Sopharma 2008) and there are no reports of cytisine metabolites in animals.

As for any other smoking cessation medications available on the market (NRT, bupropion and varenicline), the quit rates reported with cytisine use are relatively low (Vinnikov, Brimkulov et al. 2008, West, Zatonski et al. 2011) and ways to increase the effectiveness of these drugs should be investigated. Combination pharmacotherapy has been discussed as a strategy to increase smoking

abstinence in cigarette smokers. This involves using at least two drugs usually with different mechanisms of action (e.g. nicotine replacement therapy with bupropion; or varenicline with bupropion) (Ebbert, Hays et al. 2010). If cytisine is to be used with other smoking cessation drugs, its potential for DDIs needs to be explored. Preliminary studies should first investigate whether cytisine would be a substrate for enzymes responsible for the clearance of these drugs.

In general, the main pathways for drug metabolism include reactions where non-polar molecules are converted into polar compounds that can be more readily excreted and/or conjugation reactions involving groups such as glucuronic acid (Asha and Vidyavathi 2010). Cytochrome P450 (CYP) is a major enzyme involved in oxidation reactions whilst glucuronidation catalysed by UDP glucuronyl transferase (UGT) is a major pathway in the formation of water-soluble substrates that can be eliminated from the body (Sanchez and Kauffman 2010).

The following sections describe the development and validation of both the HPLC-UV and LC-MS assays. The clinical application of the LC-MS assay is also described. With the developed LC-MS assay, the metabolism of cytisine via oxidation or glucuronidation by human liver preparations was examined and is described in the chapter.

3.2 Objectives:

The objectives of this chapter were to:

1. Develop an analytical method that can support pharmacological studies of cytisine in humans
2. Use the developed assay to detect cytisine in human plasma and urine following cytisine administration
3. Screen for presence of oxidative or glucuronide metabolites *in-vitro* human liver experiment

3.3 Methods

3.3.1 Chemicals and Reagents

Cytisine (≥99% purity) and sulfanilamide (internal standard, IS) (≥99% purity) were purchased from Sigma Aldrich (Auckland, New Zealand). Methanol (>99%, HPLC grade, Sigma Aldrich) was used in sample preparation. LC-grade water (Millipore®, Milli-Q system) and methanol (>99%, HPLC grade,

Sigma Aldrich) were used for the mobile phase in the HPLC-UV method and LC-grade water and acetonitrile (ACN, >99%, HPLC-grade, Sigma Aldrich) were used for the mobile phase in the LC-MS method.

3.3.2 HPLC-UV

3.3.2.1 *Standard solutions*

Cytisine stock solutions for calibration standards were prepared in dimethyl sulfoxide (DMSO). Stock solution was further diluted in DMSO to give appropriate working solutions. The IS solution was prepared in DMSO at a concentration 400 µM.

Chromatographic separation was achieved on a Phenomenex Gemini C18 HPLC column (4.6 mm x 150 mm, 5 µm) with a guard column (C18, 4.6 x 10 mm, 5 µm). Mobile phase consisted of methanol and 50 mM ammonium acetate buffer adjusted to pH 6.5 (1 to 5% methanol gradient; flow rate 1.0 mL/min, pressure 120 bar), with UV monitoring of the column effluent. Wavelengths from 220 to 310 nm were monitored and quantification was performed at absorbance of 310 nm (cytisine) and 280 nm (IS). Signals areas were obtained from chromatograms using manual integration. Cytisine/IS peak area ratio was calculated as a quantitative measure to prepare calibration curves.

The assay was validated in accordance to the US FDA guidelines for bioanalytical methods validation over the range of 130 to 4150 ng on column for selectivity/specificity, precision and accuracy and linearity (US Department of Health and Human Services FDA 2001).

3.3.2.2 *Tablet cytisine (external QC)*

Ten cytisine tablets (Tabex® 1.5 mg film-coated tablets) were obtained from Sopharma Pharmaceuticals, Sofia, Bulgaria via the CASCAID study. Tablets from the same batch (Batch number 10211) were crushed, weighed and four tubes containing an equivalent weight of one tablet were prepared. To each tube, 1 mL of DMSO was added. The tubes were then vortex-mixed for 120 seconds, left to stand for 20 minutes at room temperature then centrifuged at 22000 *g* for 5 minutes. The supernatant was transferred to a fresh tube, mixed with internal standard and vortex-mixed for 120 seconds. An aliquot (10 µL) was then injected onto the HPLC column to determine the amount of cytisine on the column and to calculate the amount of cytisine present in one tablet. The value

was then compared to the Quality Certificate (Sopharma) documentation (analytical certificate No 324/ 17.03.2011).

3.3.2.3 *Oral strip cytisine (external QC)*

Five cytisine oral strips were obtained from an Australian marketer – Quit4Good (www.quit4good.com.au) via Dr Natalie Walker. Each strip was dissolved in 1 mL DMSO then processed as described above.

Cytisine stock solutions for calibration standards were prepared in ACN: 45 mM formate buffer, pH 4.5 (20:80, v/v). Stock solution was further diluted in ACN:formate buffer (20:80, v/v) to give appropriate working solutions. The stock solution of the IS was prepared in methanol and the working solution (400 µM) was prepared in methanol. Standard samples for the calibration curve of cytisine were prepared in DMSO. The final concentrations of cytisine in standard samples were 1.5, 3, 6, 12, 24, 48, 95, 190, 380, 760 and 1522 ng/mL. Samples used for calibration were prepared on the day of the analysis.

3.3.3 LC-MS

3.3.3.1 *Standard solutions*

Cytisine stock solutions for calibration standards were prepared in ACN:formate buffer (20:80, v/v). Stock solution was further diluted in ACN:formate buffer (20:80, v/v) to give appropriate working solutions. The stock solution of the IS was prepared in methanol and the working solution was prepared at a concentration 400 µM in methanol. Standard samples for the calibration curve of cytisine were prepared in human plasma. The final concentrations of cytisine in standard plasma samples were 1.5, 3, 6, 12, 24, 48, 95, 190, 380, 760 and 1522 ng/mL. Plasma samples used for calibration were stored in -80°C until analysis.

3.3.3.2 *Chromatographic separation*

Chromatography was achieved using an Agilent 1100 liquid chromatography (LC) system coupled with an Agilent MSD model D single stage quadrupole mass spectrum (MS) detector. Agilent ChemStation software (Version B.04.03-SP2) (Agilent Technologies, Goettingen, Germany) was used

to access processed data and chromatograms. Chromatographic separation was achieved on a Phenomenex Gemini C18 HPLC column (4.6 mm x 150 mm, 5 µm) with a guard column (C18, 4.6 x 10 mm, 5 µm). A mobile phase of 50 mM ammonium formate buffer, pH 4.5 (solvent A) and acetonitrile (solvent B) with a phase gradient 1% (B) from 0 to 3 minutes, 10% from 3 to 9 minutes and 1% at 10 minutes was used for separation. MS detection using electrospray ionisation (ESI) was performed. Detection by selective ion monitoring (SIM) (positive ion mode) for each mass ion was used: m/z 191 and 173 for cytisine and IS respectively. Drying gas flow was 12.0 L/min and the nebuliser pressure was 35 psig. The total run time was 10 minutes with a flow rate of 0.5 mL/min and sample injection size was 15 µL. Areas of signals were obtained from chromatograms using manual integration.

3.3.3.3 *Validation procedures*

The assay was validated in human plasma in accordance to the US FDA guidelines on bioanalytical method over the concentration range of 2.97 to 3043.84 pg on column for selectivity/specificity, precision and accuracy plus linearity (US Department of Health and Human Services FDA 2001).

Selectivity/specificity was examined by using blank plasma samples collected from seven different individuals to look for any endogenous peaks that could interfere with peaks for cytisine and IS. Intra- and inter-day accuracy and precision were evaluated by analysing quality control (QC) samples at 4 concentration levels: low QC, 2 mid QCs and high QC (1.5, 24, 48, 1522 ng/mL). Five replicates were evaluated per concentration. Accuracy was calculated by comparing the measured concentration with the true concentrations spiked in plasma. Precision, expressed as relative standard deviations (RSD, %), were calculated on three separate days. Calibration standards of 10 concentrations in the range 1.5 to 1522 ng/mL were prepared in human plasma and analysed (n=3) in three separate analytical runs. Calibration curves included a blank sample (no IS), a zero sample (plasma spiked with IS) and 10 non-zero samples including the limit of quantification (LOQ). LOQ was evaluated based on signal to noise ratio of 5:1 with precision and accuracy within 20% of the nominal value. Linearity was assessed by preparing calibration curves plotting the peak area ratios of cytisine to IS against the concentrations of cytisine.

Absolute recovery was assessed by comparing the peak areas of cytisine obtained from extracted spiked plasma standards with peak areas from un-extracted standards in ACN:formate buffer (20:80%, v/v).

Short term temperature stability of cytisine in plasma was examined by using QC samples (n=3) and comparing freshly spiked plasma samples to the same samples left at room temperature for 24 hours. Freeze-thaw stability was also studied by comparing freshly prepared spiked plasma samples to the same samples that were kept at of -80 °C for 24 hours and thawed at room temperature and samples that underwent 3 freeze-thaw cycles.

For urine, selectivity/specificity was examined by using blank urine samples collected from six different individuals to look for any endogenous peaks that could interfere with peaks for cytisine. LOQ was evaluated with precision and accuracy within 20% of the nominal value.

3.3.4 Sample handling and preparation

Blood samples were allowed to stand at room temperature for 30 minutes prior to centrifugation at 3000 *g* for 10 minutes to separate the plasma and red blood cell fractions. Plasma samples were stored frozen at -80° C until analysis. Aliquots (100 µL) of plasma samples were thawed at room temperature and IS (10 µL of 400 µM) was added along with ice-cold methanol (2:1 v/v). Samples were vortex-mixed for 120 seconds and left overnight at -20 °C to precipitate protein. Samples were then centrifuged (15 minutes at 22000 *g*) and 200 µL of the clear supernatant was removed and evaporated to dryness (SC210A SpeedVac® Plus, medium drying rate). The dry extract was then reconstituted with 30 µL ACN:formate buffer (20:80, v/v), centrifuged (5 minutes at 22000 *g*) and 15 µL of the final extract was injected onto column.

Urine samples were stored at -20 °C until analysis. Prior to processing, the samples were thawed at room temperature and 0.5 mL of urine sample was taken, IS was added, and diluted (1:1, v/v) with MilliQ® water. Samples were vortex-mixed and centrifuged for 10 minutes at 22000 *g*. Solid phase extraction (SPE) column (Alltech Prevail C18) was conditioned with 0.5 mL methanol then equilibrated with 0.5 mL MilliQ® water under vacuum. After loading the sample, the column was washed with 0.5 mL of methanol:water (5:95, v/v) and dried under full vacuum for 10 minutes. Methanol (0.5 mL) was used to elute the compounds of interest and the methanol eluate was collected and 15 µL was injected.

3.3.5 Clinical application

One healthy volunteer took a single 3 mg oral dose (two Tabex® tablets) to investigate whether the assay could be used to quantify drug concentrations in blood plasma and urine. Blood samples (6 mL) were collected in heparinised tubes immediately prior to dosing (*t=0*), and then at 2 hours post-dose. Urine was also collected up to 390 minutes after dosing. Samples were processed and analysed as described above.

3.3.6 *In-vitro* metabolism experiment

3.3.6.1 *Materials*

In addition to the chemicals and reagents listed in Section 3.3.1, nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Applichem, Germany. Uridine 5'-diphosphoglucuronic acid (UDPGA) and polyoxyethylene 20 cetyl ether (Brij 58) were obtained from Sigma, USA.

3.3.6.2 *Sample preparation and analysis*

A stock solution of bovine serum albumin (BSA) (20 mg/mL) was used to make an 8 mg/mL standard solution in 0.1 M sodium hydroxide (NaOH). This solution was then serially diluted with 0.1 M NaOH to generate 4, 2, 1, 0.5, 0.25, 0.125, 0.065 mg/mL BSA standards (1:1 dilution). A 5 µL aliquot of these solutions were transferred onto a 96-well plate. A blank sample (0.1 M NaOH) was also included.

Pooled human liver samples were taken from the Auckland liver bank. All samples were stored at -80 °C prior to use. The five liver preparations (all 1 mg/mL) were pooled by combining 200 µL aliquots of each.

A sample of the pooled microsomes was diluted 1:10 with 0.1 M NaOH and then this sample was serially diluted (1:1) to get a series of reducing concentrations. Aliquots (5 µL) of these solutions were transferred onto a 96-well plate. BioRad D_c Protein Assay Reagent A (25 µL) and BioRad D_c Protein Assay Reagent B (200 µL) were added to each sample well. The 96 well plate was then gently agitated for 20 minutes using a Thermomixer Comfort shaking heating block (Eppnedorf, Germany) and read on a Molecular Devices SPECTRAmax PLUS384 microplate reader (MDS Analytical Technologies, UK) at 750nm. The protein concentration was calculated using the SoftMax Pro

software. The mean protein concentration was calculated to be 25 mg/mL. The final concentration of the pooled microsomes was 20 mg/mL.

Microsome preparations (20 mg/mL) were incubated for 1 hour in a shaking water bath at 37° C with 10 mM cofactor (NADPH) and 10 mM cytisine in a final volume of 200 µL by adding 10 mM phosphate buffer, pH 7.4. The reaction was then terminated by addition of two volumes of ice-cold methanol. Samples were vortex mixed for 120 seconds and left overnight at -20 °C to precipitate protein. Samples were centrifuged for 10 minutes at 22000 *g* the next day and the clear supernatant was collected. The supernatant (50 µL) was then injected onto the HPLC column.

To examine *in-vitro* glucuronidation, microsome preparations (20 mg/mL) were incubated with 5 mM UDPGA and 10 mM cytisine in a shaking water bath at 37° C for 1 hour in a final volume of 200 µL by adding 0.1 M Tris-HCl buffer, pH 7.4 containing 5 mM $MgCl_2$ and 0.05% w/v Brij 58). The reaction was terminated by addition of two volumes of ice-cold methanol. Samples were left overnight to precipitate protein. Samples were vortex mixed for 120 seconds and left overnight at -20 °C to precipitate protein. Samples were centrifuged for 10 minutes at 22000 *g* the next day and the clear supernatant was collected. The supernatant (50 µL) was then injected onto the HPLC column.

Clozapine was used as positive control for the experiment. Samples were analysed by mass spectrometry in the scan mode (100-1000) and total ion current (TIC) was obtained.

3.4 Results

3.4.1 HPLC-UV method

Linearity was verified for this method by visual inspection and using values for coefficients of determinations (R^2) obtained from nine linear standard curves generated on three separate days. Correlation coefficients (R^2) of calibration curves were all above 0.99. Intra-day and inter-day accuracy and precision values in the range 130 to 4150 ng on column were less than 15% of the actual values.

3.4.2 Cytisine tablets and oral strips

From the calibration curve generated, cytisine concentration was calculated to be 1.43 mg/mL, corresponding to 1.43 mg per tablet. The deviation from the value reported on the Quality Certificate (1.41 mg) was 1.27% which was within acceptable limits.

The amount of cytisine in the each of the oral strips was also calculated. All strips had on average 1.00 to 1.07 mg of cytisine. The average amount of cytisine in the five oral strips was calculated to be 1.03 mg. Although no QC documentation is available, this is within 3% of the stated 1 mg/strip.

3.4.3 LC-MS

3.4.3.1 *Method validation*

Spiked plasma samples showed a symmetrical peak for both cytisine and IS. The retention times for cytisine and IS were 7.6 and 9.0 minutes respectively (Figure 3.1 and Figure 3.2). Selectivity/specificity was examined by comparing chromatograms of blank plasma samples with spiked plasma samples. No peaks were observed at the retention time of cytisine and IS in blank plasma samples collected from seven different individuals. The RSD (%) of instrument response of the different sources of blank plasma was within 15% in the seven independent plasma samples tested.

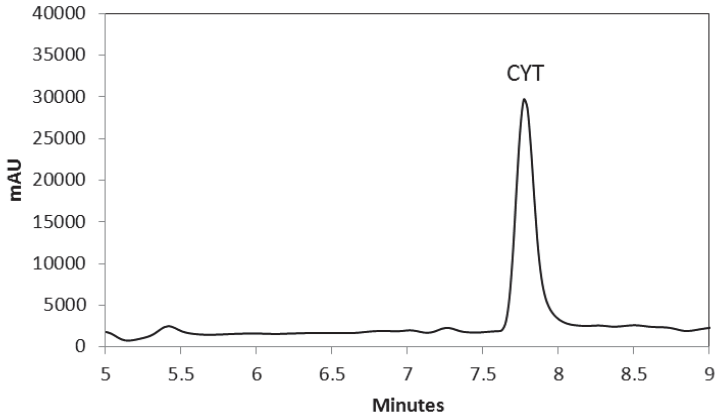

Figure 3.1. Chromatogram of plasma sample obtained at two hours after a 3 mg dose of cytisine (CYT). Detection with SIM (m/z 191).

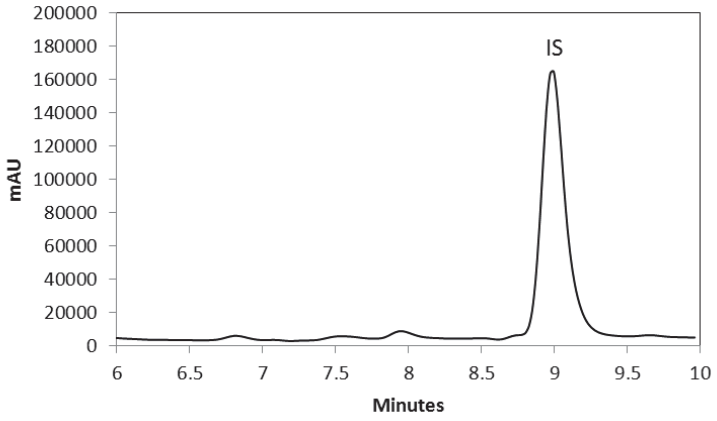

Figure 3.2. SIM (m/z 173) of zero sample chromatogram showing peak for IS.

Accuracy and precision were assessed using QC samples and values are reported in Table 3.1 and Table 3.2. Variation for intra-day and inter-day accuracy and precision was less than 15% for QC samples. Linearity was verified for this method by using values for coefficients of determinations (R^2) obtained from nine linear standard curves generated on three separate days. Correlation coefficients (R^2) of calibration curves were all above 0.99. The limit of quantification (LOQ) for cytisine for this method is 2.97 pg on column.

The absolute recovery of cytisine was consistent and on average 75% for the QC samples. Stability of cytisine in spiked plasma samples after storage in room temperature for 24 hours and after 3 freeze-thaw cycles was examined and presented in Table 3.3.

No endogenous peaks were observed at the retention time of cytisine in blank urine samples collected from six different individuals. The LOQ in urine was 152 pg on column.

Table 3.1. Intra-day variation between spiked plasma samples (n=5).

Spiked concentration (ng/mL)	Mean concentration (ng/mL)	Accuracy (%)	Precision (RSD, %)
1.5	1.4	95.3	7.6
24	23.6	99.2	11.6
48	50.4	105.9	1.3
1522	1518.6	99.8	2.2

Table 3.2. Inter-day variation between spiked plasma samples (n=5) analysed on 3 separate days.

Spiked concentration (ng/mL)	Mean concentration (ng/mL)	Accuracy (%)	Precision (RSD, %)
1.5	1.9	90.2	9.4
24	23.9	92.4	9.4
48	51.1	104.2	3.4
1522	1529.8	100.5	2.3

Table 3.3. Recovery of cytisine from spiked plasma samples after storage in room temperature for 24 hours and after 3 freeze-thaw cycles.

Spiked concentration (ng/mL)	Recovery after 24h in room temperature (%)	3 Freeze-thaw cycles (%)
1.5	91.9	89.6
24	93.0	90.9
48	99.2	93.0
1522	90.3	87.0

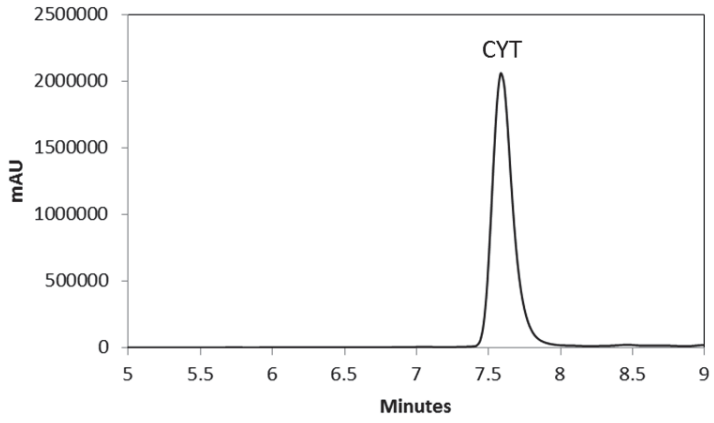

Figure 3.3. Chromatogram of urine sample collected at 300 minutes after a 3 mg dose of cytisine (CYT). Detection with SIM (m/z 191).

3.4.4 Clinical application

The plasma sample collected from the volunteer immediately prior to cytisine administration (*t=0*) did not show any endogenous interference peaks for cytisine or IS. The LC-MS assay was able to detect cytisine in the plasma samples collected at 2 hours post dose following a single 3 mg dose (Figure 3.1). Cytisine concentration measured at 2 hours was 23.38 ng/mL. Spontaneous urine samples were collected just before dosing and then at 0-90, 90-180, 180-300 and 300-390 minutes post-dose. Cytisine was detectable in every urine sample collected (Figure 3.4). The least amount of cytisine was detected in the 0-90 minute urine sample (signal intensities correspond to amount of cytisine). The greatest concentration of cytisine was detectable in the urine sample collected between 90 and 180 minutes post-dose. After 180 minutes, the amount cytisine detected in in the urine was decreased in the 180-300 minutes and further decreased in the sample collected at 300-390 minutes.

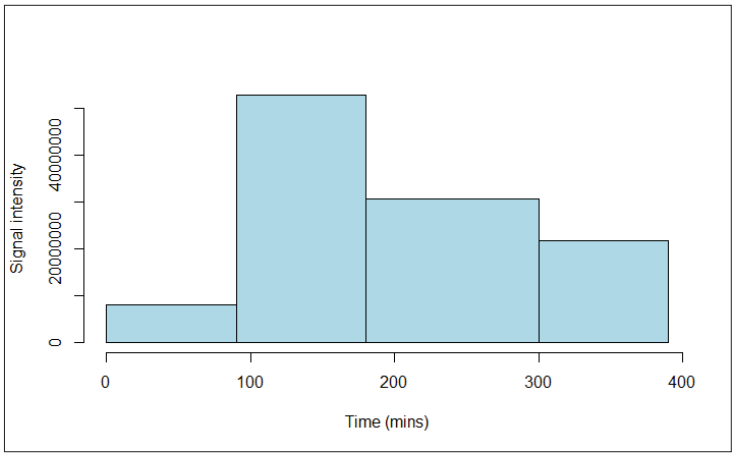

Figure 3.4. Signal intensities for cytisine in urine between 0 and 390 minutes.

3.4.5 *In-vitro* metabolism

No oxidised or glucuronide metabolites were detected in samples following the *in-vitro* metabolism experiment. For clozapine (positive control), the metabolic profile identified from the *in-vitro* experiment was consistent with literature reports (Pirmohamed, Williams et al. 1995).

3.5 Discussion

There are currently limited data on the pharmacokinetics of cytisine. The animal data that exist describe some pharmacokinetic parameters in rabbits and mice, but the doses studied are not relevant to human exposure (Klocking, Richter et al. 1980, Astroug, Simeonova et al. 2010). No published studies have measured cytisine concentrations in human plasma or urine. A validated analytical assay was required that could be applied to future clinical studies of cytisine. Although both HPLC-UV and LC-MS methods are commonly used in the detection and quantification of drugs in biological fluids, a HPLC-UV method was developed and validated first as it had the advantages of being a relatively simple and low cost procedure.

The HPLC-UV assay developed had acceptable intra- and inter-assay accuracy and precision, plus it was accurate in determining the amount of cytisine in two commercial forms of cytisine including Tabex® tablets and oral strips. This method was deemed to be externally valid. The main objective of method validation, however, is to show that the method can be used for its intended purpose with acceptable reliability and reproducibility. The true usefulness (fit for purpose) of the assay (for pharmacokinetic analysis in humans) was determined by obtaining a “real” sample, that is, a plasma sample collected from an individual after a therapeutically-relevant dose of cytisine. Only after analysing the plasma sample collected following a 3 mg single dose administration was it revealed that the HPLC-UV method was not suitable for the quantification of cytisine with dosages used for smoking cessation. Therefore, a more sensitive method was required.

The developed LC-MS analytical method for the determination of cytisine in human plasma is more sensitive compared to both the HPLC-UV method described in this chapter and the previously published analytical method (Astroug, Simeonova et al. 2010). This new method was found to comply with limits set by US FDA guidelines including accuracy, precision, specificity and linearity (US Department of Health and Human Services FDA 2001). This method has been successfully used to quantitatively determine the concentration of cytisine in a human participant following a single 3 mg dose of cytisine at 2 hours post-dose. This method, therefore, has the sensitivity required to study the pharmacokinetics of cytisine in human smokers with clinically relevant doses.

Animal studies have shown that cytisine is renally eliminated (Klocking, Richter et al. 1980). Consistent with this, this study demonstrates that cytisine is renally eliminated in humans and detectable in urine.

No metabolites were detected following *in-vitro* incubations commonly used to assess the metabolic stability of compounds. This suggests that cytisine may not be disposed to DDIs through competition for hepatic enzymes. However, its use still may have important implications for the concurrent use of other medications which are metabolised in the liver. Prolonged use of cytisine may indirectly affect the pharmacokinetics of concomitantly administered drugs. This is because chemical constituents such as polycyclic aromatic hydrocarbons in cigarette smoke can induce several enzymes and accelerate the metabolism of some drugs, particular those primarily metabolised by CYP1A2 (Kalow and Tang 1991, Schrenk, Brockmeier et al. 1998). This can lead to lower than predicted drug concentrations due to faster clearance and smokers may require higher doses of such drugs than non-smokers to achieve the desired concentration and therapeutic effect. When smoking is reduced or stopped, there is a reduction in enzyme induction and therefore there is an increase in the concentration of such drugs due to the decline in the rate of metabolism. Thus a person may experience increased adverse effects when they stop smoking, despite taking the same dose prescribed prior to smoking cessation. Given that several antipsychotic drugs, such as clozapine and olanzapine, are metabolised primarily by CYP1A2 (Bertilsson, Carrillo et al. 1994, Shirley, Hon et al. 2003), this would be particularly important for patients with schizophrenia, a population with a high incidence of smoking (de Leon and Diaz 2005). It has been shown that cigarette smoking plays a role in CYP1A2 induction that leads to reduced clozapine concentrations (Haring, Meise et al. 1989, Hasegawa, Gutierrez-Esteinou et al. 1993) and stopping smoking can lead to severe clozapine-related side effects due to increased clozapine concentrations (McCarthy 1994, Meyer 2001).

As cytisine does not appear to undergo glucuronidation, it may be a more favourable option over nortriptyline for smoking cessation since the 10-hydroxy metabolites of nortriptyline are primarily eliminated via glucuronidation (Nordin and Bertilsson 1995). Cigarette smoking has been associated with increased glucuronidation, resulting in an increased rate of elimination of drugs such as oxazepam and lorazepam (Greenblatt, Divoll et al. 1980, Fleischmann, Remmer et al. 1986, Bock, Schrenk et al. 1994).

3.6 Summary and conclusions

Commercial forms of cytisine used for smoking cessation include an oral tablet form (Tabex®) and buccal strips (nicotine-free oral strips). To date, no studies have reported cytisine concentrations in humans following use of these products. Two validated analytical assays of cytisine have been described. An HPLC-UV method was developed and validated for analysis of Tabex® and nicotine-

free oral strips. However, this assay was not sensitive enough to quantify cytisine in human plasma after clinically relevant doses. The LC-MS bioanalytical assay was therefore developed to support pharmacological studies of cytisine in humans. This method has been validated and results are within the acceptable range as set by the US FDA guidelines. This method was successfully used to detect and quantify cytisine in human plasma and urine after a single oral administration of Tabex® and could be used in the pharmacokinetic analysis of cytisine in humans. This assay was used to screen for oxidised or glucuronide metabolites in samples following incubation with human liver preparations *in-vitro*. No metabolites were detected in this study.

The next chapter will present the results from the single dose administration study of cytisine in healthy smokers.

Chapter 4 Single-dose study of cytisine in heathy smokers

4.1 Introduction and overview

Despite the commercial product (Tabex®) being available since 1960s (Paun and Franze 1968), the currently recommended dosing regimen for cytisine is complex with no apparent evidence base with respect to clinical effectiveness. The success of pharmacologically-based smoking cessation therapy is dependent on the pharmacodynamics (e.g. receptor target, selectivity/specificity; efficacy) of the drug and the concentration at the target site. The concentration achieved is dependent on various processes: absorption, distribution, metabolism and excretion. Although there have been reports in mice and rabbits (Klocking, Richter et al. 1980, Astroug, Simeonova et al. 2010), the pharmacokinetic properties of cytisine in humans have not yet been described.

An understanding of pharmacokinetic parameters can provide information on how drugs need to be dosed in individuals. This knowledge can aid rational, safe and effective use of medications, especially in special populations such as in participants with impaired renal function. Pharmacokinetic information may, therefore, be used to develop a rational dosing regimen for cytisine, or to provide evidence to support the current dosing regimen.

This chapter presents the findings of a single dose pharmacokinetic study of cytisine in seven human participants who were smokers, following a single 3 mg dose of cytisine. Whilst this involved a higher dose (double the recommended dose taken at one time) it was selected to increase the chance of detecting the drug using the analytical method described in Chapter 3.

Urine samples collected from participants were analysed on the LC-MS to detect the presence of major metabolites.

4.2 Objectives

The primary objectives of the study were to:

1. Measure the concentration of cytisine in the plasma over a 24 hour period after a single dose (3 mg cytisine) administration
2. Determine the pharmacokinetic parameters (oral clearance and apparent volume of distribution) and half-life of cytisine in humans

3. Screen for the presence of metabolite(s) in human plasma and urine

The secondary objectives were to investigate some effects of a single dose of cytisine in human participants. Specifically to measure the effect of cytisine on:

1. Heart rate, blood pressure and breathing rate, and any side effects in smokers over 24 hours
2. Craving for cigarettes and on mood following a 3 mg single dose of cytisine

4.3 Methods

4.3.1 Study design

This study involved a single dose administration of cytisine in smokers. A single dose of 3 mg was chosen to be studied in healthy smoking participants.

4.3.2 Study drug

Cytisine was given as an oral tablet form (Tabex®). Tabex® tablets were obtained from the principal investigator in the CASCAID (Cytisine as a Cessation Aid) (Walker, Howe et al. 2011) study, who in turn obtained them from Sopharma Pharmaceuticals, Bulgaria (via Extab Corporation, England). Quality Assurance (QA) was provided by Sopharma and external validation of cytisine content was also carried out (Chapter 3). All tablets were stored at room temperature away from sunlight until dispensed to participants.

4.3.3 Recruitment

Individuals who were current smokers at the time were recruited through advertising in the University of Auckland via notices placed on noticeboards throughout the University, word of mouth, personal contacts and University of Auckland Faculty of Medical and Health Sciences email lists. Interested individuals contacted the investigator through email or a telephone number. Individuals who contacted the researchers received a copy of the participant information sheet (PIS) (Appendix 3) that contained information about cytisine and details about the study via email or post (Figure 4.1).

4.3.4 Inclusion and exclusion criteria

Study participants were required to be current smokers aged 18 years and over. To be eligible participants had to: (1) have read and understood the Participant Information sheet (Appendix 3), and (2) be able to provide a written consent (Appendix 2). Participants were excluded from the study if they: (1) were pregnant or breastfeeding, (2) suffered a stroke, heart attack or severe angina in the last two weeks, (3) were diagnosed with severe/uncontrolled hypertension, (4) were diagnosed with schizophrenia, (5) were diagnosed with phaeochromocytoma, (6) had severe renal impairment (see Section 4.4.5.2), (7) only smoked non-cigarette tobacco products (e.g. pipes, cigars), (8) were a current user of nicotine replacement therapy (NRT) products, (9) were currently using non nicotine-based medicines to aid smoking cessation (bupropion, clonidine, nortriptyline, varenicline), and (10) were enrolled in another smoking cessation programme or another cessation study.

4.3.5 Screening

In order to minimise risk to participants, an initial screening by telephone was carried out to assess eligibility which included yes/no responses to the inclusion and exclusion criteria (for phone screening procedure refer to Appendix 4). Participants were asked if they were taking any medications. This information and test results were passed to the study Medical Officer who examined them and confirmed the eligibility of the participants.

4.3.5.1 *Smoking status - cotinine validation*

At screening, NicAlert® was used to confirm self-reported smoking status. NicAlert® is an immunoassay that is commercially used to test for cotinine in biological samples. A NicAlert® kit contains a NicAlert® test strip, a funnel for saliva deposit and a 2 mL tube container for collection of saliva samples. Individuals were asked to provide saliva that filled at least one third of the tube provided. The saliva was then transferred directly onto the NicAlert® strip. The strip was then left on the bench until the red area of the strip moved up into the white area above it and bands appeared on the strip. The strip was read once the blue band of the strip had disappeared. Usually within 20 minutes, a red band appeared in at least one of the zones labelled Level 0 to 6. The lowest level that a red band is detected indicated the cotinine levels detected in saliva. A level of 1 or higher in saliva indicated the use of tobacco products. The cotinine equivalents for each level of NicAlert® are indicated in Table 4.1.

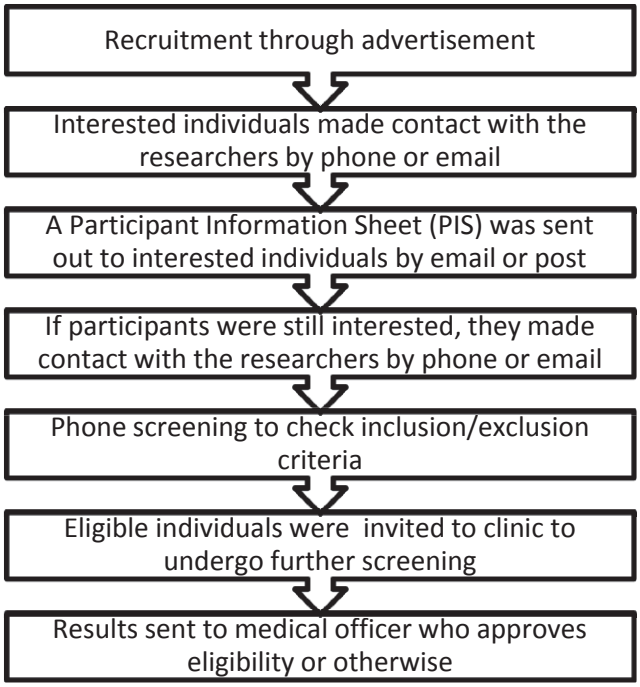

Figure 4.1. Summary of the recruitment-screening process.

Table 4.1. Cotinine concentrations for each NicAlert® level

NicAlert® Level	Cotinine concentrations (ng/mL)
0	1 – 10
1	10 – 30
2	30 – 100
3	100 – 200
4	200 – 500
5	500 – 2000
6	>2000

Source: http://www.accutest.net/products/ds47ny150.php

4.3.5.2 *Kidney function*

Kidney function was also determined at screening by a blood test and urine dipstick test. A blood sample was collected by the phlebotomist and sent off to LabTests (Auckland, New Zealand). Results (creatinine level and eGFR) were requested by the medical officer of the study who forwarded the test results to the investigator. A urine dipstick test was performed using Bayer Multistix® which tests for leuokocytes, nitrite, protein, glucose and blood. Fresh urine samples were collected in a clean 500 mL tube and tested within 10 minutes of collection at room temperature. The test area of the dipstick was immersed completely in the urine sample once and removed immediately. The reagent test strip areas were then compared to the colour chart provided on the side of the dipstick bottle. The results were read at 30 seconds for glucose, 60 seconds for protein and nitrite and 2 minutes for leukocytes (according to the instruction leaflet). Individuals had to show "negative" or close to "negative" (very small traces) of leukocytes, blood, nitrite, protein and glucose in order to be included in the study. Results were forwarded to the medical officer for examination.

4.3.6 Study setting

All participant visits were at a research-based clinic at the University of Auckland Tamaki Campus. A phlebotomist was present at the clinic to take blood samples and a nurse was present at the clinic at all times. A Medical Officer was available on call during the study. Participants were permitted to smoke but they had to walk outside the gates of the University if they wanted to (University is a smoke-free area). Participants were provided with food and beverages.

4.3.7 Sample collection and handling

4.3.7.1 *First participant*

The first study participant received two Tabex® tablets and had blood samples collected over the course of 48 hours. Blood samples were collected immediately prior to dosing (*t=0*) and then at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 24 and 48 hours post-dose. Spontaneous urine samples were collected. Blood samples were analysed using the analytical assay described in Chapter 3 to determine the concentrations of cytisine in plasma. As there were no published pharmacokinetic data on cytisine in humans (e.g. half-life), blood samples were collected up to 48 hours to determine appropriate plasma sampling times.

4.3.7.2 *Remaining participants*

On Study day 1, a blood sample was taken from each participant prior to dosing with cytisine. Nine further blood samples were collected at 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours post dosing (participants left the clinic after 8 hours and returned the next morning 24 hours post-dose). All blood samples (6 mL) were collected in heparinised tubes and allowed to stand at room temperature for 30 minutes prior to centrifugation at 3000 *g* for 10 minutes to separate the plasma and red blood cell fractions. Plasma samples were then stored at -80 °C until analysis.

Each participant provided a urine sample prior to dosing and then spontaneous samples were collected over 8 hours and then 24 hours post dose. The time was recorded each time a urine sample was collected. At each urine collection, an aliquot (10 mL) of urine was retained, labelled and placed in a -20 °C freezer immediately following collection.

On the day of the analysis, plasma and urine samples were thawed at room temperature and then handled according to the procedure outlined in Chapter 3 (Section 3.3.4).

4.3.8 Instrumental Analysis

Plasma samples were analysed using a validated method (Chapter 3) with an Agilent ChemStation liquid chromatography system coupled with mass spectrometry (LC-MS) following deproteinisation with methanol. Cytisine was resolved using a Phenomenex C18 (4.6 mm x 150 mm, 5µm) column and eluted with a mobile phase of 50 mM ammonium formate buffer, pH 4.5 (solvent A) and acetonitrile (solvent B). A solvent gradient (1-10%) was utilized for the separation with a flow rate of 0.5 mL/min. Detection was carried out by mass spectrometry and single ion monitoring (SIM) with a fragmentor voltage of 70 V for the mass ion of cytisine (191 m/z) and IS (173 m/z).

Individual and mean plasma cytisine concentrations were plotted over time and used to obtain the maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}).

4.3.9 Estimation of pharmacokinetic parameters

Non-linear, mixed effects modelling (NONMEM®) is a gold standard pharmacometrics software tool (Keizer, Karlsson et al.) that can be used to model population pharmacokinetics and was used to estimate population pharmacokinetic parameters (clearance and volume of distribution)

4.3.10 Physiological measures

Participants were seated for approximately 10 minutes before any measurements were taken. Blood pressure (systolic and diastolic, mmHg) and heart rate were measured simultaneously using an electronic blood pressure monitor (Omron Healthcare Co., Ltd., Japan, Model: SEM-2 (HEM-7051-C1)). Respiratory rate was measured by counting the number of breaths (the number of rise and fall of the participant's chest) taken by participants in one minute. Respiratory rate was measured directly after blood pressure and heart rate. All measurements were taken in triplicates.

4.3.11 Self-reported measures

All questionnaires were self-completed by participants. Paper and pencil questionnaires were used and thus scoring had to be undertaken by the researcher. Excluding the Profile of Mood States, all questionnaires are available free online. Copies of all questionnaires can be found in the Appendix (Appendix 5).

4.3.11.1 *Nicotine dependence*

The Fagerström Test for Nicotine Dependence (FTND) questionnaire (Heatherton, Kozlowski et al. 1991) was administered to individuals at screening to assess the severity of nicotine dependence. The FTND is a validated tool that contains six questions that assess the severity of nicotine dependence. The sum of the scores of each answer can range from 0 (low dependence) to 10 (high dependence). Participants had to score at least 1 to be eligible to take part in the study.

4.3.11.2 *Cigarette craving*

Craving for cigarettes was measured using the Brief Questionnaire on Smoking Urges (QSU-brief) (Cox, Tiffany et al. 2001). The QUS-brief contains ten statements to assess craving for cigarettes at the time when the questionnaire is administered. Each statement is rated on a scale of 1 (strongly disagree) to 7 (strongly agree). Total scores can range from 10 (no cravings) to 70 (severe craving). The QSU-brief was used in the study to investigate momentary craving.

Participants completed the questionnaire at 0 (before dosing), 1, 2, 3, 4, 6, 8 and 24 hours post dose. This involved participants answering 10 questions regarding the urge to smoke by asking them to

rate their strength of agreement on a 7-point scale ranging from 1 (strongly disagree) to 7 (strongly agree).

4.3.11.3 *Mood*

The Profile of Mood States (POMS) (McNair, Lorr et al. 1971) was administered to participants at 0, 2, 4, 8 and 24 hours post dose to determine five negative mood states (tension, depression, anger, fatigue and confusion) and one positive mood state (vigour). The POMS consists of 64 words to describe feeling. For each word, participants were asked to rate on a scale of 1 (not at all), 2 (a little), 3 (moderately), 4 (quite a bit) to 5 (extremely) that best described how they were feeling "right now". The scores for each word was then summed up in a specific manner (only revealed to investigators) to get scores for each separate mood subscales. Possible scores for tension are between 0 – 36; depression is between 0 – 60; anger 0 – 48, fatigue 0 – 28, confusion 0 – 28 and vigour between 0 – 32. A higher score indicated a greater feeling for the above measures. Total mood disturbance (TMD) score was calculated by adding the negative mood subscale scores and subtracting the positive mood subscale score. A higher total score indicated a greater mood disturbance.

4.3.12 Safety

Safety was assessed by clinical observation and self-reporting of side effects by participants.

4.3.13 Restrictions on smoking or diet

Participants were allowed to smoke as usual and did not have any restrictions on diet during the study, and no records were kept on their smoking or diet.

4.3.14 Data management

All questionnaire information was directly entered onto an electronic database by creating a library in SPSS. Paper copies of case report forms (CRFs- see Appendix 6) were filed in the participant's study folder and locked away in a filing cabinet.

4.3.15 Ethical approval and consent

Ethical approval was applied for and granted by the Health and Disability Ethics Committee (Northern X Regional) (NTX/11/05/038). In addition, Standing Committee on Therapeutic Trials (SCOTT) approval was obtained (ref: TT50-8671 (1219) for the clinical studies in this research as cytisine is an unlicensed product in New Zealand. Ethics and SCOTT approval letters can be found in the Appendix (Appendix 1).

4.4 Results

4.4.1 Study participants

Seven participants took part in the study (Table 4.2). Participants were all males, with a mean age of 26.3 years. On average, participants had been smoking for 9.5 years and only 2 participants had previously made an attempt to quit smoking. The average Fagerström Test of Nicotine Dependence score was 3 (moderate dependence).

Table 4.2. Participant characteristics.

Participant characteristics	**N=7**
Male, %	100
Age, mean ± SD, yrs	26.3 ± 6.58
Smoking history, mean ± SD, yrs	9.5 ± 6.98
Previously tried to quit, %	28.6
FTND score, mean ± SD	3 ± 2.08

Abbreviations: Fagerström Test of Nicotine Dependence (FTND), years (yrs)

4.4.2 Pharmacokinetics

4.4.2.1 *Cytisine concentrations in plasma*

In the first participant, cytisine was detectable in every plasma sample collected, including the early samples (15 and 30 minutes post-dose). The peak plasma concentration of 23.4 ng/mL (C_{max}) was observed at 1 hour post-dose (T_{max}). Cytisine was still detectable at 24 hours. Cytisine was not detectable in the 48 hour plasma sample. The elimination half-life of cytisine in this participant was 5

hours. This suggested that cytisine was not detectable at 48 hours because it had been completely eliminated from the body (5 half-lives removes >97% of drug from the body) and not due to the sensitivity of the assay. The sampling times selected for bloods in Participant 1 (excluding the 48 hour sample) were able to capture the absorption phase and the elimination phase of the drug and therefore deemed suitable for the study of single dose pharmacokinetics of cytisine in subsequent study participants.

Plasma cytisine concentrations were plotted over time for all participants (Figure 4.2). The peak concentrations (C_{max}) of cytisine measured in these participants were between 23.4 and 32.0 ng/mL.

Figure 4.3 shows the mean plasma cytisine concentration time profile for all participants (n=7). The mean peak plasma concentration was 27.8 ng/mL and this was reached on average at 2 hours post dose. By the end of the 24 hour sampling period, mean plasma cytisine concentrations had dropped to 0.9 ng/mL.

4.4.2.2 *Absorption*

Cytisine was detected in all plasma samples that were collected in the study, even at 15 minutes after dosing and was still detectable at 24 hours following an oral administration suggesting that it is readily absorbed. For most of the participants, C_{max} was observed at 2 hours (T_{max}) after drug administration. In two participants (participant 1 and 5), the C_{max} was observed at 1 hour after administration (Table 4.3).

4.4.2.3 *Distribution*

Log transformation of the concentration data following the peak concentration showed a single straight line which indicated that only one single elimination phase was observed (i.e. no distinction could be made from the distribution phase and the elimination phase) (Figure 4.4). For clarity, the logarithmic plot only shows the data points from when C_{max} was achieved (2 hours post dose).

4.4.2.4 *Metabolism and excretion*

Cytisine was detected in the urine as an unchanged drug; no metabolites were detected in the plasma or in urine from any of the participants in the study. Following the peak, cytisine plasma

concentrations declined in a monophasic manner. Cytisine was still detectable in the urine samples collected at 24 hours.

4.4.2.5 *Population pharmacokinetic parameters and other pharmacokinetic statistics*

The volume of distribution (V_D) and clearance (CL) values were estimated using NONMEM and values are provided in Table 4.4. Using these parameters, half-life was calculated to be 4.8 hours.

Table 4.3. Observed C_{max} and T_{max} values in participants after single 3mg dose administration.

PK statistic	Participant						
	1	2	3	4	5	6	7
C_{max} (ng/mL)	23.4	29.3	30.1	26.1	31.3	32.0	25.1
T_{max} (min)	1	2	2	2	1	2	2

Table 4.4. Estimated population PK parameters for single dose cytisine. Values shown as mean and SEM (n=7).

Parameter estimates	**Population estimate**	**SEM**
CL (L/hr)	16.7	0.003
V_D (L)	115	0.003

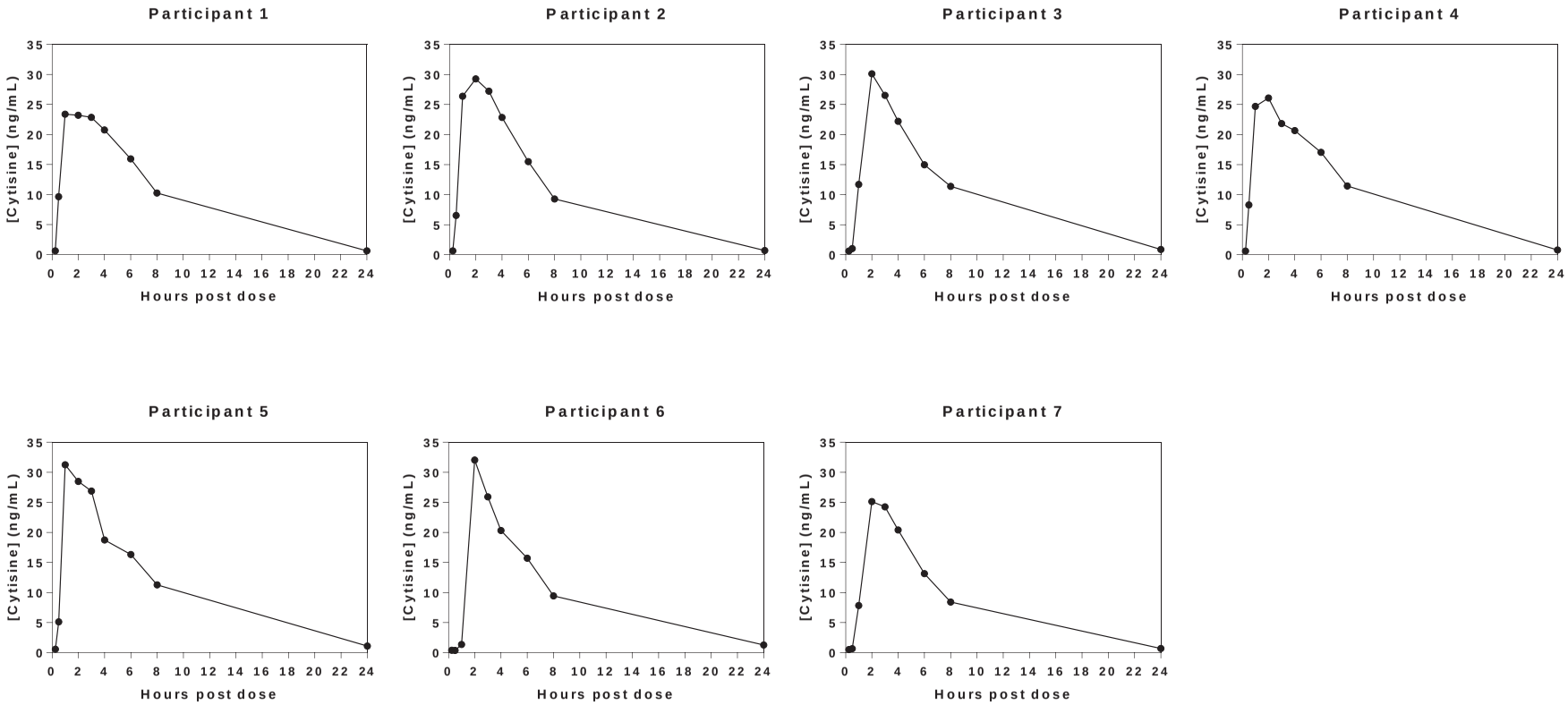

Figure 4.2. Plasma cytisine concentrations measured in 7 participants following a single 3 mg dose.

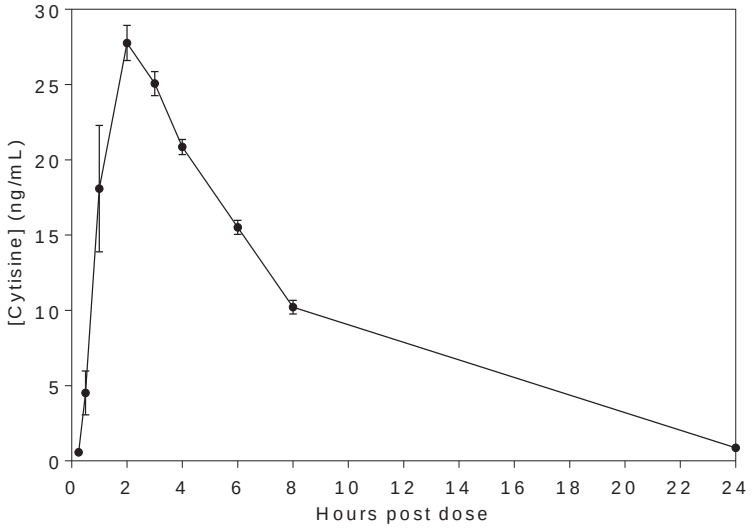

Figure 4.3: Plasma concentrations (ng/mL) of cytisine over 24 hours following a single 3 mg dose. Values are shown as mean ± SEM (n=7).

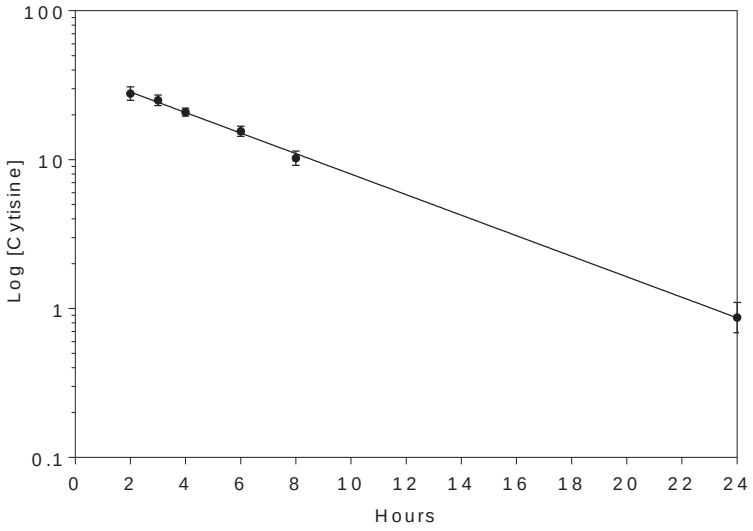

Figure 4.4: Log[Cytisine] vs. time showing a single linear elimination phase after maximum concentration is reached (2 hours). Values shown are mean ± SEM (n=7).

4.4.3 Other measurements

4.4.3.1 *Physiological measures*

Overall, blood pressure, heart rate and respiratory rate appeared to be unaffected after a 3 mg single dose administration of cytisine (Figure 4.5).

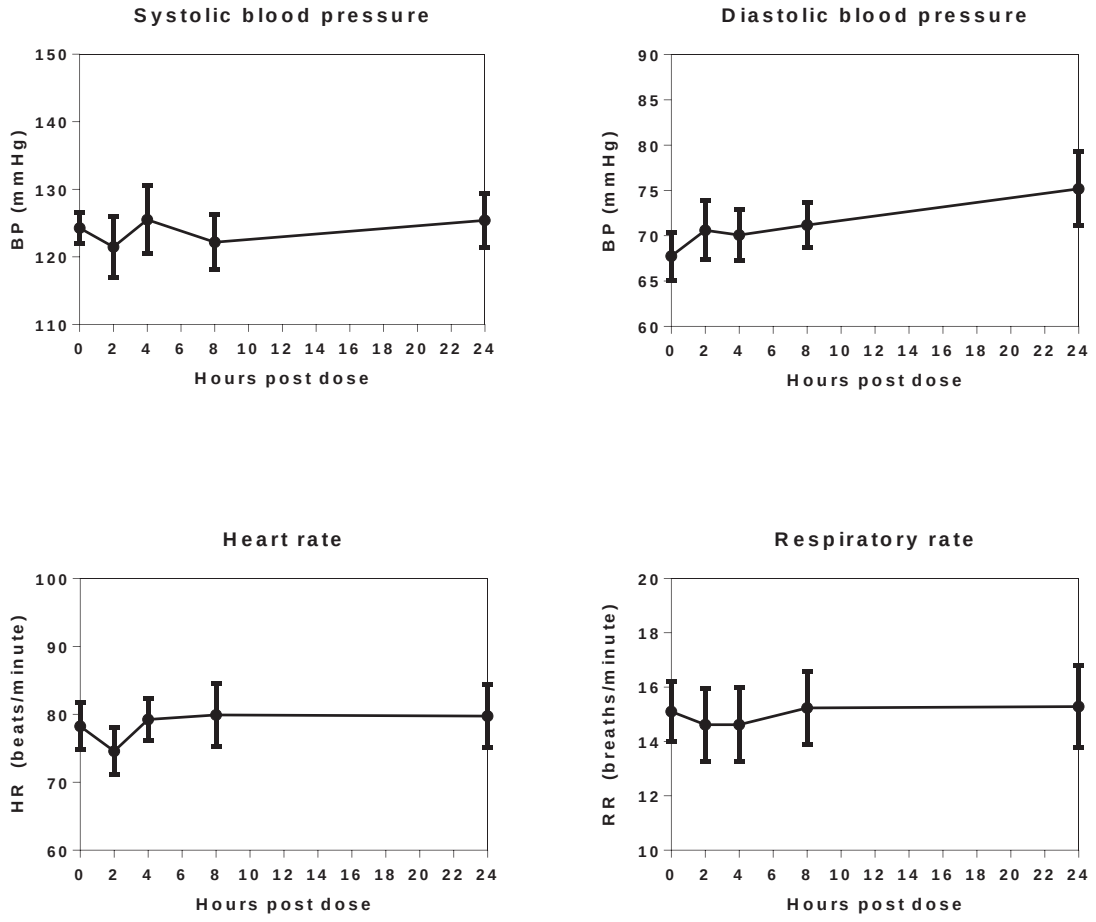

Figure 4.5: Blood pressure (systolic and diastolic), heart rate and respiratory rate following a single 3 mg dose of cytisine. Values shown are mean ± SEM (n=7).

4.4.4 Self-reported measures

4.4.4.1 *Urge to smoke*

Higher scores in the QSU-brief indicate a greater urge to smoke. QSU-brief scores against time plots show that urge scores varied across the participants at the beginning of the study (Figure 4.6). The urge scores decreased in all participants at some point during the study after 3 mg administration of

cytisine. In 4 participants, the urge to smoke remained reduced and did not return to baseline scores within 24 hours. In 3 participants, the urge scores were reduced by more than 50% at greatest compared to baseline (participants 1, 5 and 6).

4.4.4.2 *Mood*

The POMS results showed that none of the subscales measured (tension, depression, anger, fatigue, confusion and vigour) appeared to be affected by a single 3 mg dose administration of cytisine (Figure 4.7). Therefore, the total mood disturbance appeared to remain stable throughout the 24 hour period.

4.4.4.3 *Relationship between cytisine concentration and effect*

The time-course of the changes in mean urge to smoke were compared to the time course of the mean plasma concentrations of cytisine over 24 hours following a single 3 mg dose (Figure 4.8). The mean craving scores decreased after administration of cytisine. The lowest mean craving score was observed after the peak plasma cytisine concentration was achieved. It appeared that an increase in cytisine concentration coincided with a decrease in cigarette craving, but a decrease in cytisine concentration did not appear to directly affect the craving scores that were already reduced. Therefore, plasma cytisine concentration did not appear to have a direct impact on cigarette cravings. The mean craving score did not return to baseline at the end of the study and was lower than the mean score at the start of the study despite the decreased cytisine plasma concentration.

Plasma cytisine concentrations did not appear to be associated with mood changes in this study (Figure 4.7).

4.4.5 Safety

A single oral dose of 3 mg cytisine was well tolerated in all subjects. No side effects were reported by the participants during the study.

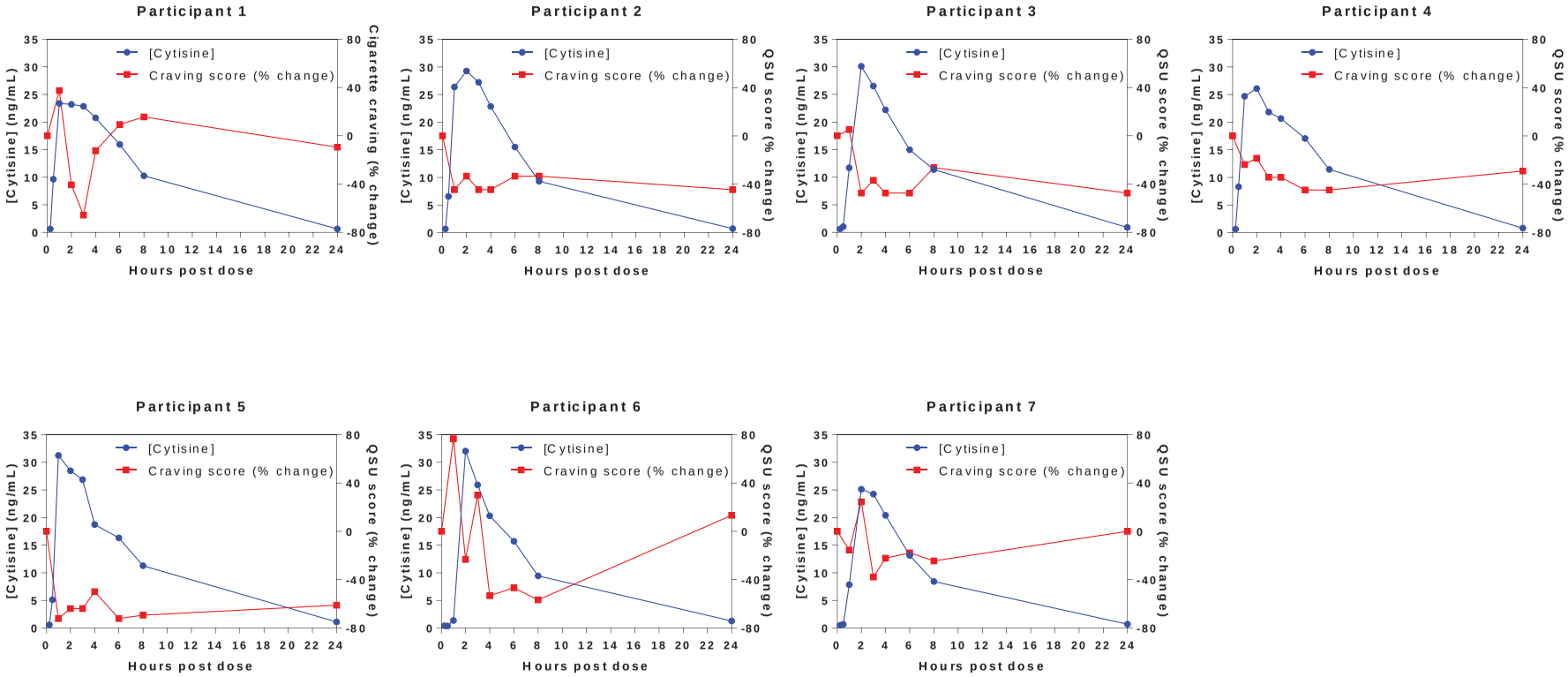

Figure 4.6: Change in cigarette craving (% from baseline), measured using QSU-brief, and plasma cytisine concentrations following a single 3 mg dose of cytisine in 7 participants.

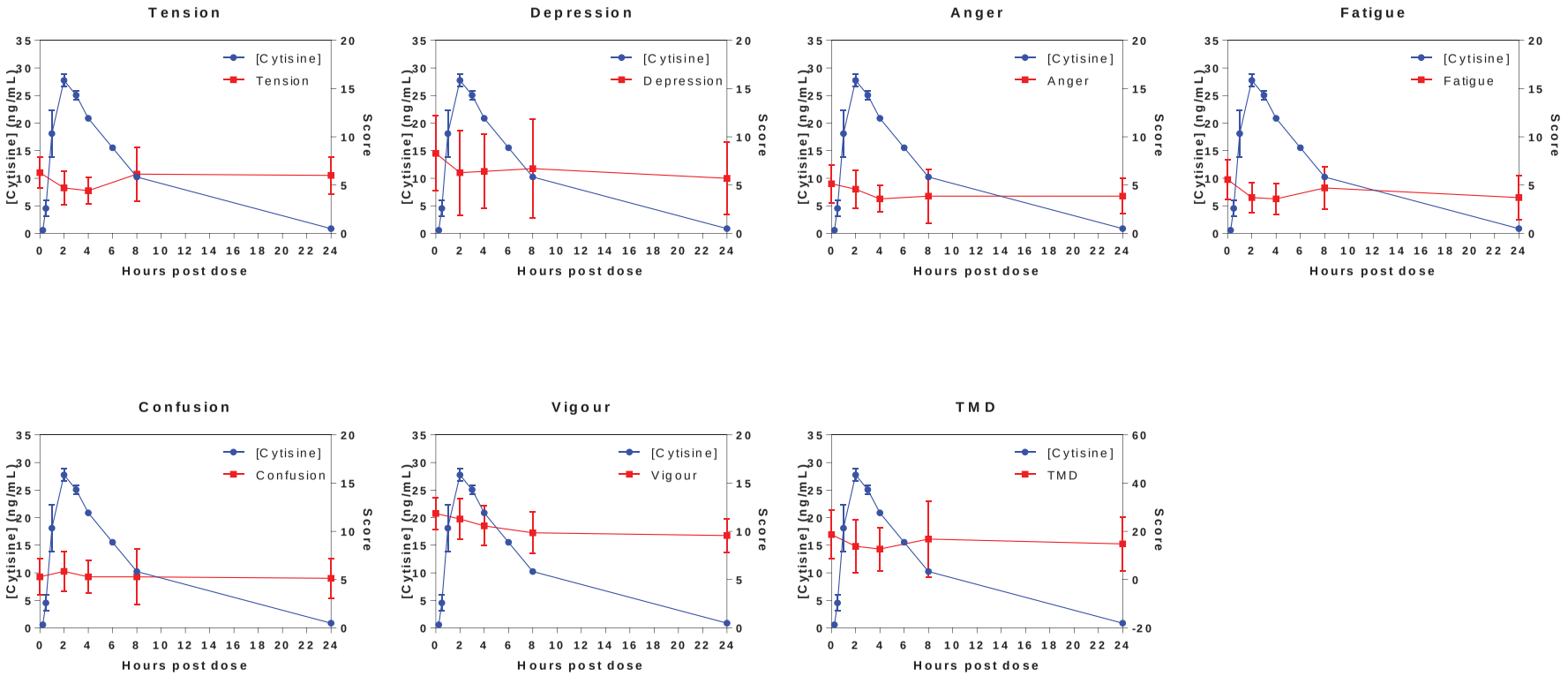

Figure 4.7: Time course plots for each of the six subscales (tension, depression, anger, fatigue, confusion, vigour), total mood disturbance (TMD) in the POMS and plasma cytisine concentrations. Values shown are mean ± SEM (n=7).

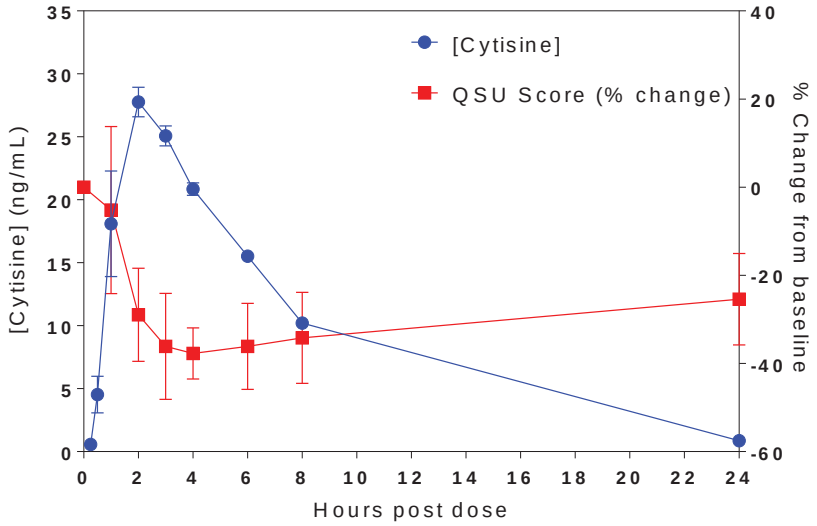

Figure 4.8: Time-course plots for plasma cytisine concentration and mean urge to smoke (determined using QSU-brief) over 24 hours after a single 3 mg dose administration of cytisine. Values shown are mean ± SEM (n=7).

4.5 Discussion

There is currently limited data on the pharmacokinetics of cytisine in animal studies, and none to date reported for humans. The doses studied in animals are not clinically relevant for humans (Klocking, Richter et al. 1980, Astroug, Simeonova et al. 2010).

It was hypothesised that cytisine would be eliminated in a similar manner to varenicline, a synthetic analogue of cytisine.

The current study is the first to describe the pharmacokinetics of cytisine in humans following a single oral 3 mg dose in healthy smokers. After administration, cytisine is absorbed into the bloodstream, with cytisine detected in plasma as early as 15 minutes after dosing. Peak plasma concentrations were typically achieved within 1 to 2 hours after administration. Apart from two participants (participants 1 and 5), the observed T_{max} was 2 hours in all of the participants. However, as no blood samples were taken between 1 and 2 hours post dose (e.g. 90 minutes post dose), the true C_{max} may actually have been achieved between these two time points for all participants.

Plasma cytisine concentrations declined with an average elimination half-life of 4.8 hours. The half-life of cytisine has been previously reported in 2 animal species. In rabbits, the half-life of cytisine

following an oral administration has been reported to be relatively short (51 minutes) (Astroug, Simeonova et al. 2010).

The logarithmic plot of cytisine concentration versus time after C_{max} (2 hours post dose) revealed a single elimination phase (i.e. no distinction could be made between the distribution and elimination phase of the drug), which was consistent for all participants in the study. This indicates that single dose pharmacokinetics of cytisine may be described using a one compartment model. This then proposes two possible explanations for how cytisine is distributed in the body. Cytisine may either (i) stay in the blood compartment or (ii) cytisine may distribute rapidly to other compartments. The apparent volume of distribution for cytisine estimated for the population in the study (115 L) is much larger than the blood compartment (35 L), which suggests that the latter explanation is more likely. However, it is unknown to which tissues/organs cytisine distributes. In addition, since the absolute bioavailability of cytisine is unknown, a large apparent volume of distribution may in fact be due to low bioavailability.

These results show that pharmacokinetics of cytisine are markedly different from varenicline. Cytisine not only has a much shorter half-life than varenicline (4.8 h vs 24 h) (Burstein, Fullerton et al. 2006), but is also different in the way it is distributed in the body. Varenicline concentrations in plasma decrease in a biphasic manner and are best described as a two-compartment model (Ravva, Gastonguay et al. 2009). In addition, the apparent volume of distribution of varenicline is approximately 3-fold greater than cytisine (Ravva, Gastonguay et al. 2009), which suggests that varenicline may be distributed to tissues more extensively. In animal models, varenicline readily crosses the blood brain barrier (Rollema, Shrikhande et al. 2010). The effect of these pharmacological agents are expected to be dependent upon the concentrations achieved in the brain (centrally acting drugs) and potencies at the target receptor ($\alpha_4\beta_2$-nAChR) and therefore it is desirable to look at the extent of brain penetration of these drugs. Unlike varenicline, which is known to readily cross the blood brain barrier (Rollema, Shrikhande et al. 2010), cytisine has been shown to have poor entry into the brain in animal models (Romano, Goldstein et al. 1981, Reavill, Walther et al. 1990, Mineur, Eibl et al. 2009, Rollema, Shrikhande et al. 2010).

In rats, a subcutaneous injection of 1 mg/kg of cytisine resulted in an average brain cytisine concentration of 145 ng/mL at 15 minutes post dose, which was less than 30% of the plasma concentration (Reavill, Walther et al. 1990). Interestingly, the acid dissociation constant (pKa) of cytisine is 7.8 (*cf*. 9.3 for varenicline) which indicates that cytisine exists in its ionised form at lower levels than varenicline at physiological pH (7.4). Therefore, ionisation alone does not explain

cytisine's limited brain penetration in animals and suggests that cytisine may be removed or excluded from the brain via active efflux mechanisms. However, cytisine does not appear to be a substrate for P-glycoprotein (P-gp) and its susceptibility to breast cancer resistance protein (BCRP) transporters does not differ significantly from varenicline (Rollema, Shrikhande et al. 2010). More work is needed to explore whether cytisine is a substrate for other active efflux transporters. Cytisine, however, is potent and binds to $\alpha_4\beta_2$ nAChRs at nanomolar concentrations (Ki values ranging from 0.45- 2.4 nM) (Boido, Tasso et al. 2003, Gonzales, Rennard et al. 2006, Jorenby, Hays et al. 2006, Rollema, Shrikhande et al. 2010) which may suggest that even with limited blood brain barrier penetration, the exposure of cytisine at $\alpha_4\beta_2$ nAChRs in the brain may be sufficient to result in biologically significant activation of these receptors.

Animal studies have shown that cytisine is renally eliminated (Klocking, Richter et al. 1980). Consistent with the hypothesis, the current study demonstrates that cytisine is renally eliminated in humans and readily detected in urine like varenicline. Furthermore, consistent with the findings from the *in-vitro* metabolism study (Chapter 3), no metabolites of cytisine were detected in the urine samples collected. The metabolism of cytisine has not been studied extensively, but preclinical studies have found that cytisine undergoes minimal metabolism with 90-95% of the administered dose excreted unchanged in the urine (Sopharma 2008) and animal studies in rabbits did not report the presence of metabolic products (Klocking, Richter et al. 1980). For varenicline, two minor metabolites have been identified in human urine (hydroxyquinoxaline and *N*-carbamoylglucuronide metabolites), but more than 90% of the administered dose in the blood and urine is unchanged varenicline (Obach, Reed-Hagen et al. 2006). As with varenicline, metabolism appears not to be a primary route of elimination for cytisine, in contrast to bupropion and nortriptyline (other drugs used for smoking cessation) which are extensively metabolised by hepatic enzymes (Venkatakrishnan, von Moltke et al. 1999, Hesse, Venkatakrishnan et al. 2000). For example, bupropion is extensively metabolised to three active metabolites (hydroxybupropiom, threohydrobupropion and erythrohydrobupropion) (Hsyu, Singh et al. 1997). These reactions are catalysed primarily by CYP2B6, but other CYP isozymes are also involved including CYP1A2, CYP2A6, CYP2C9, CYP2E1 and CYP3A4 (Schroeder 1983, Hesse, Venkatakrishnan et al. 2000). As such, there is potential for interactions to occur with drugs that affect the CYP2B6 enzyme. Even if cytisine is metabolised, its metabolites will be present in very low concentrations compared to cytisine and so it is unlikely that the metabolites will be pharmacologically active. Hepatic insufficiency is, therefore, unlikely to lead to changes in the pharmacokinetics of cytisine and it is unlikely that cytisine will have clinically-relevant drug-drug interactions (DDIs) due to competition for hepatic enzymes. This may

offer an advantage over the use of bupropion and nortriptyline because they are extensively metabolised by CYP enzymes (Venkatakrishnan, von Moltke et al. 1999, Hesse, Venkatakrishnan et al. 2000) and thus more disposed to interactions with drugs that are substrates for these enzymes. However, as discussed in Chapter 3, drug interactions can exist due to smoking and smoking cessation.

On the other hand, as cytisine is eliminated primarily through renal clearance, renal insufficiency would need to be explored to determine whether renal impairment leads to increased systemic exposure to cytisine and a prolonged half-life in plasma, and if so, whether this leads to increased adverse effects. For varenicline, severe renal impairment leads to 2.1-fold increase in area under the curve (AUC) and reduced dosing is recommended for these participants (Pfizer 2014). In addition, if cytisine clearance involves active renal secretion (transporters), there is potential for DDI with other renally secreted drugs that are substrates for the same transporters. Varenicline is excreted partially via active renal secretion and has been shown to be a substrate for human organic cation transporter 2 (hOCT2) but not for other major renal transporters such as the human organic anion transporters (hOAT1 and hOAT3) and human organic cation/carnitine transporters (hOCTN1 and hOCTN2) (Feng, Obach et al. 2008). As cytisine would exist partly as cations at physiological pH, it is expected that the drug would also be a substrate for active renal transport involving organic cation transporters.

Overall, cytisine administration did not appear to affect blood pressure, heart rate and respiratory rate despite the dose used being double the recommended to be ingested at one time (Sopharma 2008). No side effects were reported and 3 mg of cytisine was well-tolerated in all participants. The most common feature of cytisine toxicity reported in the literature (both animal studies and clinical studies) includes distresses in the gastrointestinal (GI) tract such as nausea/vomiting (Tutka and Zatonski 2006, Zatonski, Cedzynska et al. 2006, Vinnikov, Brimkulov et al. 2008, West, Zatonski et al. 2011), although a meta-analysis found no significant difference between cytisine and placebo in humans (Hajek, McRobbie et al. 2013). Nausea is a commonly reported dose-related adverse effect with the use of varenicline (Gonzales, Rennard et al. 2006, Faessel, Ravva et al. 2009, Pfizer 2014). A study found that varenicline is less well-tolerated under fasting conditions, and nausea and vomiting may be reduced when varenicline is taken with food (Faessel, Smith et al. 2006). It would be interesting to explore whether this is the same for cytisine as this study was done with non-fasting participants.

Psychological effects were also measured in the study, particularly for cravings for cigarettes and mood. A decrease in cigarette craving was observed in all participants after single dose administration of cytisine. The results however, did not demonstrate a clear relationship between cytisine concentration and cigarette craving. In addition, the reduced craving scores did not add significant meaning to the effects of cytisine as participants were allowed to smoke during the study and this would have affected the responses in the questionnaire data. The mood scores measured by POMS showed that a 3 mg single dose of cytisine did not appear to result in significant mood disturbance. Again, this may have been also affected by cigarette smoking.

A limitation of this study was that although there was no exclusion for sex, all participants were male. Future studies on female smokers are needed to determine whether sex-related differences in kinetics exist. In addition, future studies will need to look at cytisine pharmacokinetics in renally impaired patients and other special population groups to determine whether dose adjustments should be made to promote safe use of cytisine. The participants in this study were relatively homogenous in terms of sex and build (all relatively fit and no one was overweight) which may explain why there was very little variation in the drug concentration-time profiles between different participants. In addition, the study population consisted of relatively light smokers (indicated by FTND smokers) and most of the participants were not intending to quit smoking. This may have affected the results in the questionnaire data.

Another limitation of this study was that no data were collected on the effect of food or drink on the pharmacokinetics of cytisine. As metabolism is not of concern for cytisine, effect of foods on absorption of cytisine would be an area to explore. Given its water solubility and renal elimination, the use of diuretics such as caffeine or alcohol would be predicted to have an effect on the pharmacokinetics of cytisine.

More recently, a buccal strip containing cytisine has been marketed in Australia and is available to purchase online on several sites (http://www.quit4good.com.au/, http://www.quitnoeffort.com/, http://www.quitnowhelp.com/). Interestingly, cytisine is not a registered medication in Australia and no Quality Assurance (QA) documentation of these products are available. Each strip is said to contain 1 mg of cytisine so there is less cytisine than in the tablets but the recommended dosing regimen (25-day course) is exactly the same. A striking difference between the two formulations is the price – the price for a 25-day full course can be between $US 189-300 (http://www.quitnoeffort.com/, http://www.quitnowhelp.com/). Buccal delivery allows drugs to be absorbed directly into systemic circulation avoiding degradation in GI tract and first pass metabolism

(Şenel and Hıncal 2001). However, the advantages of using this route for cytisine are uncertain (given the expense) as it is not extensively metabolised and appears to be readily absorbed following oral administration. It would be interesting though to study the bioavailability of these two different dosage forms of cytisine to determine the differences in the absorption profile of the two formulations and, more importantly, whether this has an impact on cravings for cigarettes. However, as there is no QA documentation of the oral strip products available, it is uncertain whether these products contain only cytisine.

The next logical investigation of cytisine would be to investigate the multi-dose pharmacokinetics of cytisine to determine whether there is drug accumulation, whether there are changes to the pharmacokinetics of cytisine over the dosing timeframe and whether there are any changes to the metabolism (induction) of cytisine in humans. More importantly, investigation into the relationships between cytisine concentration and its effects should be fully explored. Multi-dose pharmacokinetics, cytisine's effects on craving for cigarettes, withdrawal, mood and smoking satisfaction over the recommended dosing period will be presented in the next chapter.

4.6 Summary

Single dose pharmacokinetics of cytisine were studied in 7 healthy adult male smokers who took 2 tablets of Tabex® (one tablet contains 1.5 mg of cytisine). Cytisine was well tolerated: heart rate, blood pressure and respiratory rates remained stable throughout the study and no safety concerns were identified at this dose. Cytisine was detected in all plasma samples collected in the study, including 15 minutes and 24 hours post dose. In summary, cytisine showed a simple pharmacokinetic profile with maximum concentrations reached between 1 and 2 hours post dose and an estimated elimination half-life of 4.8 hours. Cytisine was renally excreted and detected as unchanged drug.

As cytisine does not appear to undergo significant hepatic metabolism, it appears that it would be unnecessary to reduce the dose of cytisine for individuals with impaired hepatic function. However, renal function may be an important factor that affects cytisine concentration.

The next chapter will present the results from the multi-dose study involving smokers who were instructed to adhere to the recommended dosing regimen of Tabex®.

Chapter 5 Cytisine as a smoking cessation aid: measuring drug levels, craving, withdrawal, mood and smoking satisfaction during the recommended dosing regimen.

5.1 Introduction and overview

The previous chapter has described the pharmacokinetics of cytisine in healthy male smokers following a single 3 mg dose. As noted previously, the current standard dosing regimen of Tabex® (a commercially available form of cytisine) is complex and differs markedly from the dosing regimen for varenicline (a synthetic analogue of cytisine developed for smoking cessation). The usual starting dose for varenicline is 0.5 mg once daily during titration. The dose is increased to 0.5 mg twice daily on day 4 then from day 8, the recommended dose is 1 mg twice daily, which is maintained for 12 weeks (Ebbert, Wyatt et al. 2010). In contrast, the recommended dosing regimen of Tabex® is more intense over a much shorter treatment period (25 days) and involves administering tablets more frequently per day for most of the treatment period. The initial dose (for the first three days) is 6 Tabex® tablets per day with a 2 hour interval between each tablet (9 mg cytisine in total). This is followed by a gradual dose tapering over the remaining treatment period (detailed dosing regimen is described under Methods in this chapter). There is also a designated quit day (day 5) for smokers who are following this dosing regimen. This dosing regimen is, however, without any published rationale.

A complex dosing regimen may be associated with poor adherence to therapy that may ultimately affect the effectiveness of the treatment (Claxton, Cramer et al. 2001). Studying the pharmacokinetics of a drug may be one way to gain an understanding (and thus look for ways to improve) the dosing regimen. It is unknown whether cytisine concentration under recommended dosing reaches a steady state concentration, like varenicline (Faessel, Gibbs et al. 2006). Therefore it was considered worthwhile to conduct a multiple administration study to investigate whether there is drug accumulation and to study whether there are dose-dependent changes to the pharmacokinetics of cytisine. Studying the multi-dose pharmacokinetics of cytisine in a more diverse group of smokers could help to determine whether there is between-subject variability and whether any variability can be related to the effectiveness of the treatment.

Cytisine has been shown to be more effective than placebo in helping smokers quit (Cahill, Stead et al. 2012). In general, effectiveness for smoking cessation drugs is related to the subjective measures

such as craving, mood and smoking satisfaction. It would, therefore, be worthwhile to investigate the effect of cytisine on these psychological measures which will allow concentration-effect relationships to be determined. Studying the concentration-effect relationship could improve the understanding of how cytisine works as a smoking cessation agent and shed light on whether there is an optimal or threshold concentration required to produce a desired response in smokers.

5.2 Objectives and research questions

The main objectives of the study were:

1. To describe the plasma pharmacokinetics of cytisine over the time course of the recommended dosing regimen
2. To screen for presence of metabolites in urine collected from participants
3. To measure the effect of cytisine on physiological and psychological measures including craving for cigarettes, nicotine withdrawal, mood and smoking satisfaction
4. To explore whether these effects can be related to the plasma concentrations of cytisine, in participants who were instructed to follow the recommended Tabex® dosing regimen

As the findings in this chapter cover a range of domains, the results section will be presented in six different parts (A to F) that aim to answer specific research questions listed below.

Part A: Pharmacokinetics

1. Does cytisine accumulate in plasma during the recommended dosing period?
2. Does cytisine reach steady state concentration under normal dosing?

Part B: Physiological and psychological effects

1. Does cytisine affect heart rate, blood pressure and respiratory rate?
2. Does anything change from baseline to end of treatment with cytisine?
    a. Are there changes to cigarette cravings?
    b. Are there changes to the severity of withdrawal symptoms?
    c. Are there changes to mood?

3. Are there differences between smokers and non-smokers during the study?
    a. Are craving scores higher in non-smokers than smokers?
    b. Are withdrawal symptoms more severe in non-smokers than smokers?
    c. Are there greater mood disturbance in non-smokers than smokers?
    d. Are withdrawal symptoms more severe in non-smokers than smokers?
4. Do participants quit smoking on day 5? If they do not why not?
    a. Are there differences in cravings in participants who quit smoking on day 5 and those who do not?
    b. Are there differences in smoking satisfaction between participants that quit smoking on day 5 and those who do not?
5. Can we predict who will quit smoking based on subjective measures?

Part C: Patterns of smoking

1. What happens to cigarette smoking during the study?
2. Is smoking related to craving?

Part D: Concentration-effect relationship

1. Is there a relationship between plasma cytisine concentration and cigarette cravings during the recommended dosing period?
2. Is there a relationship between plasma cytisine concentration and quitting?

Part E: Validation of smoking cessation

1. How does the biochemical validation of smoking compare to self-reported smoking status?

Part F: Safety and compliance

1. What side effects were reported in the study?
2. What is the compliance with the standard dosing regimen of Tabex®?

5.3 Methods

5.3.1 Study drug

Tabex® tablets were obtained in the same way as the single dose study (Section 4.3.2). In addition, tablets were re-packaged to meet the labelling requirements of the Medicines Regulations (Regulations 1984) before they were given to participants. An information sheet about Tabex® (Tabex® package insert) was provided to every participant (Appendix 7). An inventory of the Tabex® tablets was set up and records were kept on batch number, unique participant identifier and amount dispensed to each participant. Participants were asked to return all unused tablets at the end of the study.

5.3.2 Recruitment

Recruitment was undertaken in the same way as described in the single dose study (Section 4.3.3). Individuals who were interested in participating received a Participant Information sheet (PIS) (Multi-dose study PIS, Appendix, 3).

5.3.3 Inclusion/exclusion criteria

In addition to the inclusion criteria listed in the single dose study chapter (Section 4.3.4), individuals also had to score at least “moderate dependence” in the Heaviness of Smoking Index (HSI, adapted from Fagerström Test for Nicotine Dependence (FTND)) which consist of 2 questions from the FTND (*How soon after you wake up do you smoke your first cigarette?* and *How many cigarettes/day do you smoke?*). The exclusion criteria were the same as the single dose study (Section 4.3.4).

5.3.4 Screening

An initial phone screening was undertaken as outlined in Section 4.3.5 but this time the HSI was initially administered to measure the severity of nicotine dependence instead of the FTND. Participants had to score at least 3 on the HSI to be eligible, at which point they were invited to visit the clinic. Following written consent, participants completed further screening at the clinic through questions on medications (to assess phaeochromocytoma and schizophrenia) (List of questions on medications can be found in Appendix 6). Participants were also asked about their smoking habits, i.e. the number of cigarettes smoked per day, the duration of smoking (years) and whether they had

any previous quit attempts. They also completed the rest of the FTND. Blood pressure measurements, dipstick test results, blood results and reports of any medications taken by the participant were assessed by the medical officer before finalising the eligibility of each participant. Once approved by the study Medical Officer, participants were eligible to commence the study.

5.3.4.1 *Smoking status - cotinine validation*

At screening, self-reported smoking status was confirmed as outlined in the single dose study (Section 4.3.5.1).

5.3.4.2 *Kidney function*

Kidney function was determined at screening as outlined in the single dose study (Section 4.3.5.2)

5.3.5 Study setting

All participant visits took place at a research-based clinic at the University of Auckland Tamaki Campus. A phlebotomist was at the clinic to take blood samples and a nurse was available at the clinic at all times. A Medical Officer was available on call throughout the study. Participants were permitted to walk around the clinic, lunch, snacks and beverages were provided on days 1 and 25. Although permitted to smoke, participants had to walk outside the gates of the University to smoke because the University is a smoke-free area.

5.3.6 Cytisine dosing

Each participant was provided with a full course of treatment of Tabex™ (100 tablets containing 1.5 mg cytisine). Participants were instructed to follow the standard Tabex™ dosing regimen which is: 6 tablets per day on days 1-3 (1 tablet every 2 hours), 5 tablets per day on days 4 – 12 (1 tablet every 2.5 hours), 4 tablets per day on days 13 – 16 (1 tablet every 3 hours), 3 tablets per day on days 17 – 20 (1 tablet every 5 hours) and 2 tablets per day on days 21 – 25 (1 tablet every 6 hours). To ensure participants followed the dosing regimen, dosing instructions were clearly printed on the study diary (See Section 5.3.12) and participants were encouraged to carry around the instructions of the dosing

regimen printed on a wallet-sized laminated card (handed out at the beginning of the study). Participants were asked to document any deviations from the recommended dosing regimen.

5.3.7 Sample collection and handling

On Study day 1, blood samples were taken from each participant prior to dosing with cytisine. Five further blood samples were collected at 2, 4, 6, 8, 10 hours post dosing. Blood samples were collected immediately before taking the next tablet (On day 1 one tablet is taken every 2 hours for a total of six tablets (Table 5.1). Participants went home and returned on Days 2, 3, 4, 5, 6, 13, 14, 17, 18, 21 and 22 to provide a blood and urine sample immediately before the first dose of the day. On day 25, four blood samples were collected over a 6-hour period (immediately on arrival and then at 2, 4, 6 hours after the first dose of the day). Participants went home and returned the next morning (day 26) to provide a final blood and urine sample (Table 5.2). This was a pragmatic study and all blood samples were collected before the participants took the next dose of cytisine (trough concentrations).

All blood samples (6 mL) were collected in heparinised tubes and allowed to stand at room temperature for 30 minutes prior to centrifugation at 3000 *g* for 10 minutes to separate the plasma and red blood cell fractions. Plasma samples were then stored at -80 °C until analysis.

Each participant provided a urine sample prior to dosing and then spot samples were provided at each clinic visit. Participants collected urine in the morning prior to attending the clinics. An aliquot of urine was retained, labelled and placed in -20 °C freezers for later analysis.

On the day of the analysis, plasma and urine samples were thawed at room temperature and then handled according to the procedure outlined in Chapter 3 (Section 3.3.4).

Table 5.1. Study schedule on Day 1

Time (hours post dose)	Cytisine	Blood	VS	Questionnaires			
				mCEQ	MPSS	QSU-brief	POMS
On arrival		Y	Y	Y	Y	Y	Y
0	Y						
1							
2	Y	Y	Y			Y	Y
3							
4	Y	Y	Y			Y	Y
5							
6	Y	Y				Y	
7							
8	Y	Y	Y			Y	Y
9							
10	Y	Y	Y			Y	Y

Notes: The dosing regimen for day 1 is one Tabex® tablet (1.5 mg cytisine) every 2 hours. Blood samples were taken just before the next dose. Abbreviations: Vital signs (VS, includes heart rate, diastolic and systolic blood pressure and respiratory rate), modified Cigarette Evaluation Questionnaire (mCEQ), Mood and Physical Symptoms Scale (MPSS), Questionnaire on Smoking Urges-brief (QSU-brief) and Profile of Mood States (POMS).

5.3.8 Instrumental Analysis

Plasma samples were analysed using a validated method (Chapter 3) with an Agilent ChemStation liquid chromatography system coupled with mass spectrometry (LC-MS) following deproteinisation with methanol. Cytisine was resolved using a Phenomenex C18 (4.6 mm x 150 mm, 5 µm) column and eluted with a mobile phase of 50 mM ammonium formate buffer, pH 4.5 (solvent A) and acetonitrile (solvent B). A solvent gradient (1-10%) was utilized for the separation with a flow rate of 0.5 mL/min. Detection was by mass spectrometry and single ion monitoring (SIM) with a fragmentor voltage of 70 V for the mass ion of cytisine (191.2 m/z) and IS (173.2 m/z).

Individual plasma cytisine concentrations were plotted over time.

5.3.9 Estimation of pharmacokinetic parameters

Pharmacokinetic modelling and estimation of population pharmacokinetic parameters were performed as outlined in the single dose study (Section 4.3.9).

Table 5.2. Summary of days vs interventions

Study days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Cytisine (total dose (mg) per day)	9	9	9	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	6	6	6	6	4.5	4.5	4.5	4.5	3	3	3	3	3	
Number of blood samples taken	6	1	1	1	1	1							1	1			1	1			1	1			4	1
Vital signs (HR, BP, RR)	Y	Y	Y	Y	Y	Y							Y	Y			Y	Y			Y	Y			Y	Y
Cotinine validation of smoking status	Y	Y	Y	Y	Y	Y							Y	Y			Y	Y			Y	Y			Y	Y
QSU-brief	Y	Y	Y	Y	Y	Y							Y	Y			Y	Y			Y	Y			Y	Y
MPSS	Y	Y	Y	Y	Y	Y							Y	Y			Y	Y			Y	Y			Y	Y
POMS	Y	Y	Y	Y	Y	Y							Y	Y			Y	Y			Y	Y			Y	Y
mCEQ	Y	Y	Y	Y	Y	Y							Y	Y			Y	Y			Y	Y			Y	Y

Abbreviations: Heart rate (HR), blood pressure (BP), respiratory rate (RR), Questionnaire on Smoking Urges-brief (QSU-brief), Mood and Physical Symptoms Scale (MPSS), Profile of Mood States (POMS) and modified Cigarette Evaluation Questionnaire (mCEQ). “Y” indicates that measurement took place.

5.3.10 Physiological measures

Blood pressure (systolic and diastolic), heart rate and respiratory rate were measured as outlined in the single dose study (Section 4.3.10). On day 1, measurements were made on arrival then at 2, 4, 8, 10 hours post dose (Table 5.1). Measurements were also taken at each study visit (Table 5.2).

5.3.11 Self-reported measures

Questionnaires used in the single dose study (Questionnaire on Smoking Urges-brief (QSU-brief) and Profile of Mood States (POMS)) (Section 4.3.11) were also used in the multi-dose study. In addition, several other questionnaires were also administered to participants and are described below. Copies of all questionnaires can be found in the Appendix (Appendix 5).

5.3.11.1 *Cigarette craving*

Craving for cigarettes was measured using two validated scales: the QSU-brief (Cox, Tiffany et al. 2001) (Section 4.3.11.2) as well as the MPSS (West and Hajek 2004). Unlike the QSU-brief, the urge item in MPSS was used to indicate how strong the cravings have been for the participant over the past 24 hours (*cf*. "now" in QSU-brief). Urge to smoke in the MPSS is rated from 5 (*all the time*), 4 (*almost all the time*), 3 (*a lot of the time*), 2 (*some of the time*), 1 (*a little of the time*) to 0 (*not at all*) and strength of urges are rated from 5 (*extremely strong*), 4 (*very strong*), 3 (*strong*), 2 (*moderate*), 1 (*slight*) to 0 (*no urges*).

5.3.11.2 *Nicotine withdrawal*

The MPSS (West and Hajek 2004) was also used to assess several feelings that are associated to nicotine withdrawal. Items in the questionnaire include *depressed*, *irritable, restless, hungry* and *poor concentration*. These items are rated on a scale from 1 (*not at all*), 2 (*slight*), 3 (*somewhat*), 4 (*very*) to 5 (*extremely*).

5.3.11.3 *Smoking satisfaction*

The Modified Cigarette Evaluation Questionnaire (mCEQ) was used to assess the reinforcing effects of smoking (Cappelleri, Bushmakin et al. 2007) . The questionnaire contains 12 statements that are rated on a scale of 1 (*not at all)*, 2 (*very little)*, 3 (*a little)*, 4 (*moderately)*, 5 (*a lot)*, 6 (*quite a lot)* to 7 (*extremely)*. Subscales of the questionnaire include smoking satisfaction, psychological reward, aversion, enjoyment of respiratory tract sensations and a single item rating of craving reduction from smoking a cigarette. The questionnaire can only be administered to participants who continue to smoke and thus smoking status was asked first before administering the questionnaire.

A summary of the questionnaires used are presented in Table 5.3.

5.3.11.4 *Mood*

Mood was measured using POMS as described in the single dose study (Section 4.3.11.3).

5.3.11.5 *Administration of questionnaires*

On day 1, MPSS and mCEQ were administered once on arrival. QSU-brief and POMS were administered every 2 hours (on arrival (t=0), then at 2, 4, 6, 8 and 10 hours) to coincide with blood sampling times. On Days 2, 3, 4, 5, 6, 13, 14, 17, 18, 21 and 22, MPSS, mCEQ, QSU and POMS were administered once. On day 25, MPSS and mCEQ were administered once on arrival. The brief-QSU and POMS were administered 4 times (on arrival then at 2, 4, 6 hours) to coincide with blood sampling times (Table 5.1).

Table 5.3. Summary of questionnaires used in the multi-dose study

Questionnaire	What it measures	Rating style
Questionnaire on Smoking Urges-brief (QSU-brief) (Cox, Tiffany et al. 2001)	Cigarette craving	10 items (questions) on urge to smoke at that time. Rating scale for each item is from 1 (strongly disagree) to 7 (strongly agree).
Mood and Physical Symptoms Scale (MPSS) (West and Hajek 2004)	<u>Withdrawal symptoms:</u> Depression Irritability Restlessness Hunger Poor concentration <u>Cigarette craving:</u> Urge to smoke Strength of urges	Withdrawal symptoms are rated on a scale from 1 (not at all) to 5 (extremely). Urge to smoke (in the past 24h) is rated from 5 (all the time) to 0 (not at all). Rating scale for strength of urges is from 5 (extremely strong) to 0 (no urges).
Modified Cigarette Evaluation Questionnaire (mCEQ) (Cappelleri, Bushmakin et al. 2007)	Smoking satisfaction Psychological reward Aversion Enjoyment of respiratory tract sensations Craving reduction	Rating scale from 1 (not at all) to 7 (extremely) *Completed only if you have smoked since you last completed this questionnaire
Profile of Mood States (POMS) (McNair, Lorr et al. 1971)	Total mood disturbance (TMD) and mood subscales: Tension Depression Anger Fatigue Confusion Vigour	Rating scale from 1 (not at all) to 5 (extremely) for 64 items. These scores are used to calculate a score for each subscale of mood. Total mood disturbance is the sum of all mood subscales minus vigour.

5.3.12 Study diary

A study diary (Appendix 7) was developed and provided to each participant on day 1. The study diary contained the Participant Information Sheet (PIS) (Appendix 3), Tabex® information sheet (Appendix 7) and a clear outline of the dosing regimen. The diary also contained a smoking diary (Section 5.3.13), a dosing diary (Section 5.3.14), a sleep diary (see Section 5.3.15) and a comment section (Section 5.3.16). Participants were asked to fill out the diary every day during the study and were asked to bring the diary with them to each clinic visit. Participants were asked whether they would like to receive text message reminders to fill in their diary during non-visit days.

5.3.13 Self-reported smoking and biochemical validation

Participants were instructed to record their smoking in a smoking diary. For each day of the study, participants recorded the lag time from waking to smoking the first cigarette, the number of cigarettes smoked from waking up to noon, noon to 6 pm and 6 pm to bedtime, and the total number of cigarettes smoked that day.

At each visit, one NicAlert® saliva cotinine test was used to assess smoking status of each participant (see Section 4.3.5.1 for test procedure). A NicAlert® level of 0 was used an indicator of abstinence (no nicotine exposure) whereas a NicAlert® level of 1 or higher indicated smoking (exposure to nicotine).

5.3.14 Medication adherence

Participants were asked to keep a dosing diary to record the time that each tablet was taken and to record any missed doses. Participants brought their study diaries along with the remaining Tabex® tablets each time they came back to the clinics. Medication adherence was assessed using tablet counts and examining the dosing diary. On day 1, participants were asked if they would like to receive reminder texts to fill in the daily diary. To ensure participants followed the dosing regimen, dosing instructions were printed clearly on the study diary and a wallet-size laminated card. Participants were encouraged to carry around the card in their wallets. Participants were reminded whenever the dosing frequency changed.

5.3.15 Sleep

Participants were instructed to complete the sleep section of the study diary to record the total hours of sleep, number of awakenings during the night and overall quality of sleep. All assessments were reported to the medical officer. Participants brought their study diaries each time they attended the clinics.

5.3.16 Safety assessment

Safety was assessed by clinical observation and self-reporting of side effects (either by face-to-face with the researchers or reporting using the study diary). Participants were required to record any unusual occurrences or adverse reactions for each day of the study in the study diary provided. At each visit, participants had a private session with the investigator to go through what was recorded in the study diary. Participants were verbally asked if they had any other comments to make or if they had experienced any adverse reactions or unusual occurrences that they had not noted down in the study diary.

5.3.17 Designated quit date

Participants were asked to quit smoking on day 5 (following the manufacturer's recommendations, www.tabex.net), but participants who were unable to quit on day 5 were not excluded from the study.

5.3.18 Restrictions on diet and medications

Participants did not have any restrictions on diet during the study, and no records were kept on their diet. There were no restrictions on medications apart from the medications listed in the exclusion criteria (NRT, bupropion, clonidine, notriptyline or varenicline). Participants were told to inform the investigator(s) if they had taken any medication other than cytisine.

5.3.19 Behavioural support

Participants were not encouraged (nor discouraged) to seek help from other services to help them quit smoking (e.g. Quitline). Participants were not provided with any support materials. At the end of

the study (day 26), participants were asked if they had received any additional help (counselling, Quitline or other behavioural support).

5.3.20 Data management

All questionnaire information was directly entered onto an electronic database by creating a library in SPSS. Paper copies of case report forms (CRFs- see Appendix 6) were filed in the participant's study folder and locked away in a filing cabinet.

5.3.21 Statistical considerations

As these were preliminary studies from which we hope to gain information needed for larger concentration-effect studies, calculation for study power was not required.

5.3.21.1 *Statistical testing*

Since this study had small number of participants (N=10), and the data was not normally distributed (SPSS was used to determine skewness and kurtosis), it was necessary to use a non-parametric test. Thus statistical testing (when required) was performed using Wilcoxon signed-rank test, a non-parametric equivalent of the paired *t*-test (Field 2007). $P < 0.05$ was considered statistically significant. The Wilcoxon signed-rank tests were carried out using IBM SPSS Statistics software version 22.

5.3.22 Ethical approval and consent

Ethical approval was applied for and granted by the Health and Disability Ethics Committee (Northern X Regional) (NTX/11/05/038). In addition, Standing Committee on Therapeutic Trials (SCOTT) approval was obtained (ref: TT50-8671 (1219) for the clinical studies in this research as cytisine is an unlicensed product in New Zealand. Ethics and SCOTT approval letters can be found in the Appendix (Appendix 1).

5.4 Results

5.4.1 Participants

Eleven participants (4 males and 7 females) initially took part in the study; ten of these participants completed the study. For the rest of the chapter, results of the ten participants who completed the study will be presented, unless stated otherwise. Results of the participant that withdrew from the study are presented later in the chapter (Section 5.10.2.2).

The mean age of these ten participants was 39.9 years (Table 5.4). The mean number of years participants had smoked was 23.6 years. The mean FTND score was 5.8 (high nicotine dependence). Eight of the ten participants had tried to quit smoking in the past.

Table 5.4. Baseline participant characteristics.

Characteristics	**N=10**
Male : Female	3 : 7
Mean age (range)	39.9 (21-55)
Duration of smoking, mean ± SD, yrs	23.6 ± 10.4
FTND score, mean ± SD	5.8 ± 1.5
NicAlert® level, mean ± SD	4.3 ± 0.7
Tried to stop smoking previously	8

Abbreviations: Fagerström Test for Nicotine Dependence (FTND), years (yrs)

5.5 Part A: Cytisine pharmacokinetics

5.5.1 Overview

Currently, there are no published data on the pharmacokinetics of cytisine following multiple administrations. This section will describe the pharmacokinetic findings of the study.

5.5.2 Does cytisine accumulate in plasma during the recommended dosing period?

On day 1, the mean trough cytisine concentrations increased with repeated dosing (1.5 mg every 2 hours) and accumulation of cytisine was observed in all participants. The mean ± SD plasma trough

concentration measured before the last dose of the day (at 10 hours) was 50.8 ± 14.2 ng/mL (Figure 5.1).

Variability was observed in plasma concentrations between participants even at 2 hours after the administration of the first dose; for example, one participant had more than 3 times higher concentration than another participant. This variability was observed continuously throughout day 1. For the remainder of the study, there appeared to be an increase in the mean trough plasma concentrations during the first 4 days of the treatment. However, there was minimal day-to-day accumulation of cytisine. On day 2, the mean ± SD trough concentration measured was 7.1 ± 5.6 ng/mL which was less than half of the trough cytisine concentration measured at 2 hours on day 1 (17.0 ± 5.6 ng/mL). Overall, the mean trough cytisine concentrations decreased as the study progressed due to a decrease in dosing frequency (Figure 5.2).

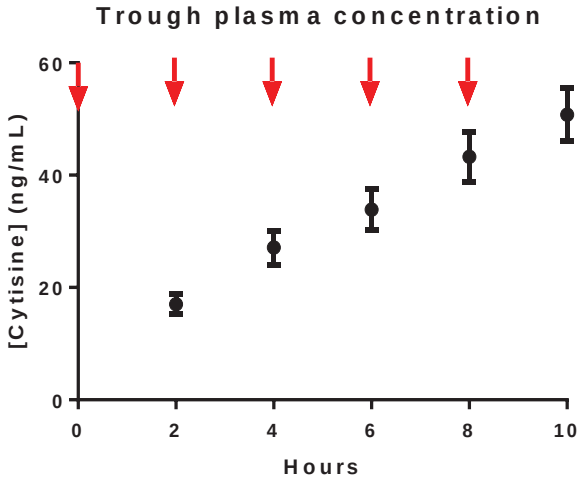

Figure 5.1: Trough concentrations of cytisine in plasma on Day 1, in 10 participants. Values are shown as mean ± SEM.

Notes: Red arrows indicate doses of cytisine (every 2 hours). All blood samples were collected just prior to taking the next dose.

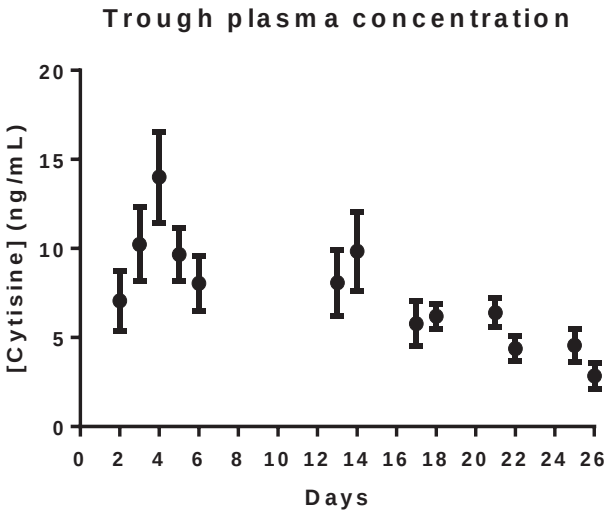

Figure 5.2: Trough concentrations of cytisine in plasma in 10 participants. Values are shown as mean ± SEM.

5.5.3 Does cytisine reach steady state concentration under normal dosing?

As described above, there was no between-day accumulation of cytisine, thus cytisine concentrations in plasma do not reach a steady state with the current recommended dosing. However, the trough concentrations measured in this study did not provide an extensive plasma concentration-time profile of cytisine over the whole recommended dosing period. Therefore, data from the single dose study (Chapter 4) was incorporated in the data from this study to model the multi-dose pharmacokinetics in these participants.

5.5.3.1 *Modelling and estimation of pharmacokinetic parameters*

The estimated pharmacokinetics parameters (Table 5.5) were very consistent with those estimated in the single dose study. Again, this suggests that there were no dose-dependent changes to the pharmacokinetics of cytisine.

Table 5.5. Estimated population pharmacokinetic parameters. Values shown as mean and SEM (n=18).

PK parameter	Population estimate	SEM
V_D	98.2 L	0.2
CL	13.8 L/h	0.2

For this chapter, the pharmacokinetic modelling of two participants will be used as examples to describe the overall concentration-time profile of cytisine during the recommended dosing period. These participants had the lowest and highest measured cytisine plasma concentrations. The concentration data for each participant can be found in the Appendix (Appendix 9).

From the modelled plasma concentrations, it is evident that variability exists between participants in pharmacokinetics such that one participant would consistently have a higher plasma cytisine concentration than another participant throughout the dosing period. Thus the population model consistently either over-predicted or consistently under-predicted the plasma cytisine concentration for participants in the study (Figure 5.3).

5.5.4 Urine analysis

Consistent with analyses of urine from others who had taken cytisine (Chapter 3 and 4), no cytisine metabolites were detected in the urine samples collected in the multi-dose study.

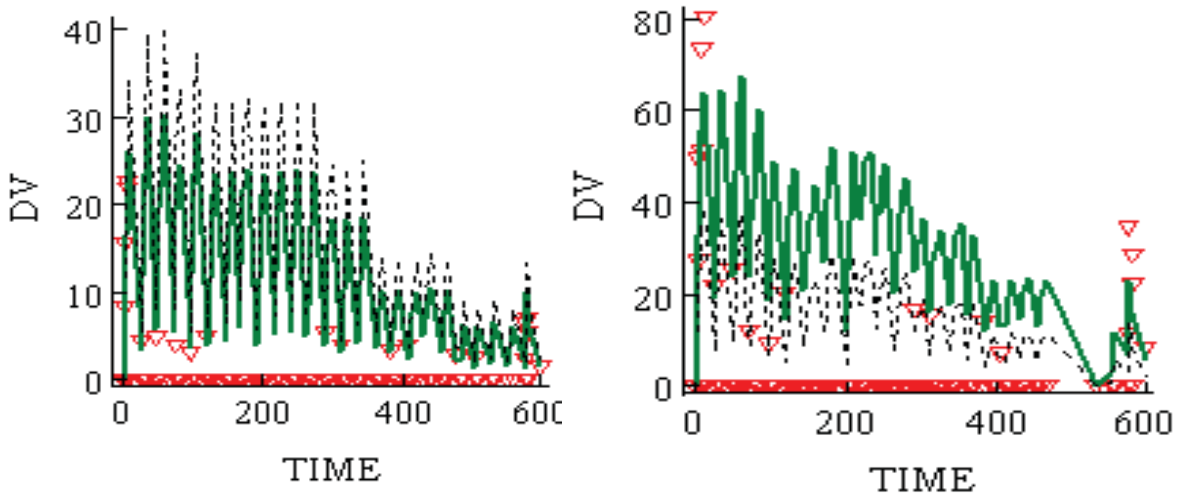

Figure 5.3: Examples of modelled cytisine concentration-time profiles for two participants during the study period.

Notes: The participant with the lowest plasma concentrations of cytisine (left panel) and highest plasma concentration of cytisine (right panel). Red inverted triangles represent the measured concentrations in the individual, the green line represents the model for the individual and the dotted line is the predicted population model. The red inverted triangles at the bottom of each graph represent the dose taken by the participant.

5.6 PART B: Effect of cytisine on physiological and psychological measures

5.6.1 Overview

This section will describe the effect of cytisine on the physiological and psychological measures measured in the study.

5.6.2 Does cytisine affect the heart rate, blood pressure and respiratory rate?

Despite the frequent dose administrations on day 1, cytisine did not appear to affect heart rate, blood pressure or respiratory rate. Overall, mean heart rate, systolic blood pressure, diastolic blood pressure and respiratory rate in participants taking the recommended doses of cytisine remained relatively stable across the 26-day study period (Figure 5.4 to Figure 5.6).

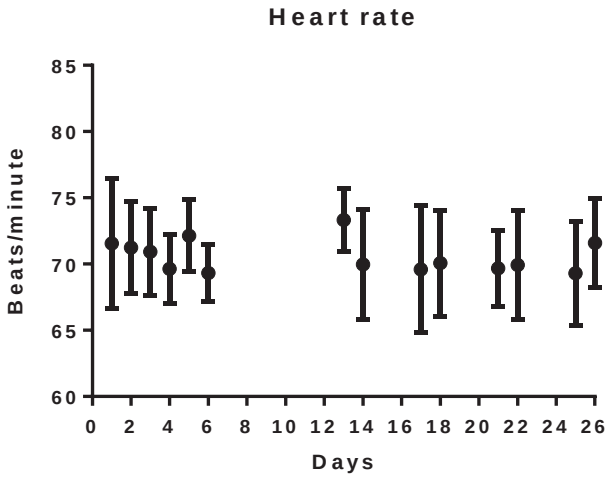

Figure 5.4. Heart rate (beats per minute) measured in participants (n=10) during the study. Values are shown as mean ± SEM.

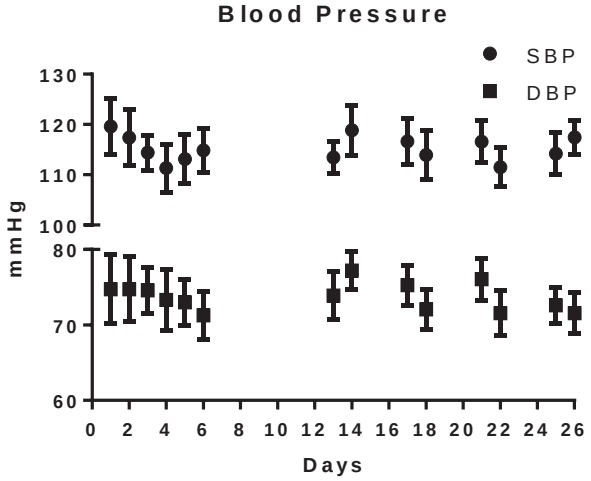

Figure 5.5: Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in participants (n=10) during the study. Values are shown as mean ± SEM.

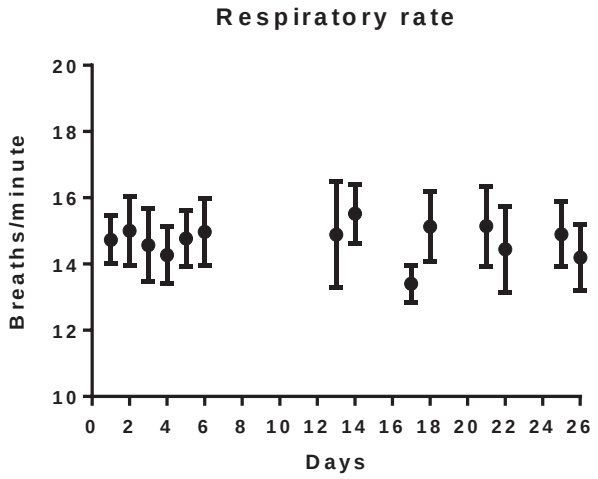

Figure 5.6: Respiratory rate (breaths per minute) in participants (n=10) during the study. Values are shown as mean ± SEM.

5.6.3 Does anything change at the end of treatment?

5.6.3.1 *Are there changes to cigarette craving?*

The mean craving scores measured using the QSU-brief declined over time (Figure 5.7). All participants reported that cigarette craving was less strong at the end of the study than before cytisine therapy (Figure 5.8). At the end of the study, QSU-brief cravings scores were significantly lower than baseline (z=-2.803, p-value=0.05). Consistent with this, both urge to smoke and strength of urge in the past 24 hours measured using the MPPS appeared to decline over time (Figure 5.9). There was a significant decline in mean urge to smoke from baseline to the end of the study (z=-2.831, p-value=0.05). Similarly, the strength of urge were significantly decreased in the study (z=-2.844, p <0.05) (Table 5.6).

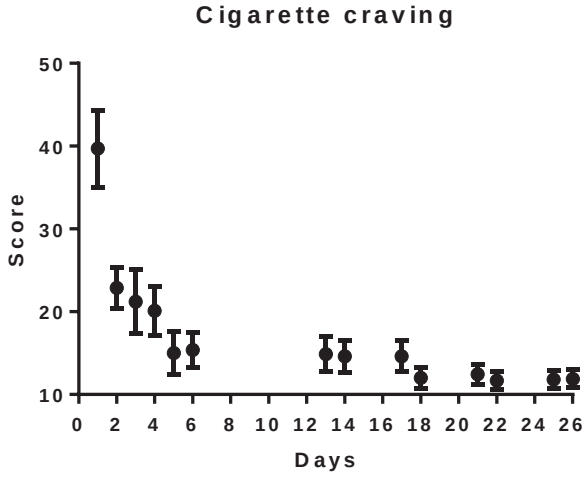

Figure 5.7: Cigarette craving scores (QSU-brief) in 10 participants. Values are shown as mean ± SEM.

5.6.3.2 *Are there changes to the severity of withdrawal symptoms?*

Overall, the mean ratings of the severity of withdrawal symptoms, including depression, irritability, restlessness, hunger and poor concentration measured using the MPPS appeared to remain relatively stable across the 26-day study period (Figure 5.10). There was no significant change in symptom severity from baseline to the end of the study period (Figure 5.10).

5.6.3.3 *Are there changes to mood?*

Overall, the mood subscales and total mood disturbance (the sum of all mood subscales minus vigour) measured using the POMS appeared to fluctuate from day to day (Figure 5.11 and Figure 5.12). However, mean scores for negative moods including tension, depression and confusion on day 26 were not significantly different from baseline. Mean scores for positive mood (vigour) at the end of the study did not significantly change from baseline. On the other hand, anger and fatigue were significantly decreased from baseline to the end of the study. There was no significant change in mood overall as the mean total mood disturbance at the end of the study was not significantly different from baseline (Figure 5.12).

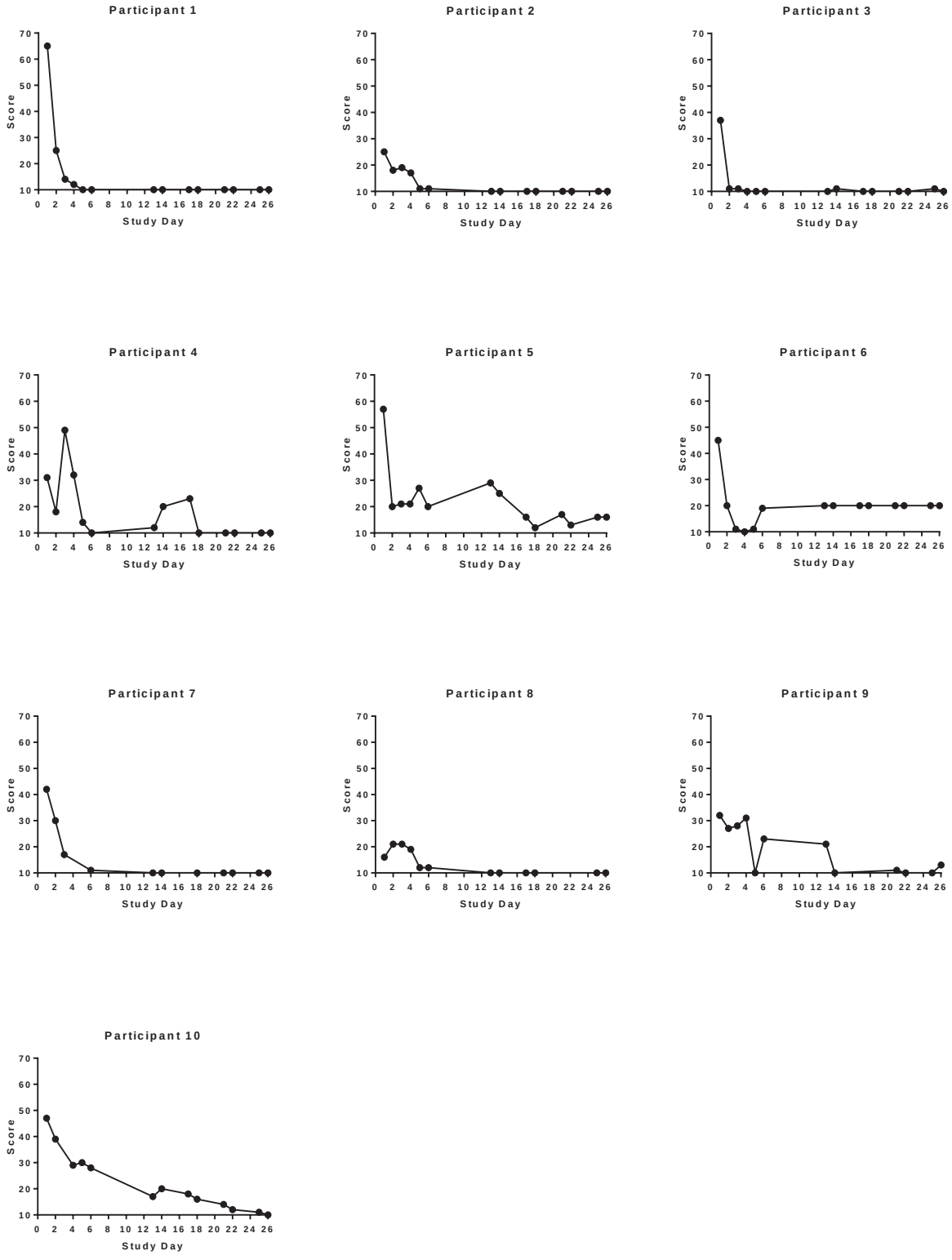

Figure 5.8: Cigarette craving scores (QSU-brief) for participants during the study.

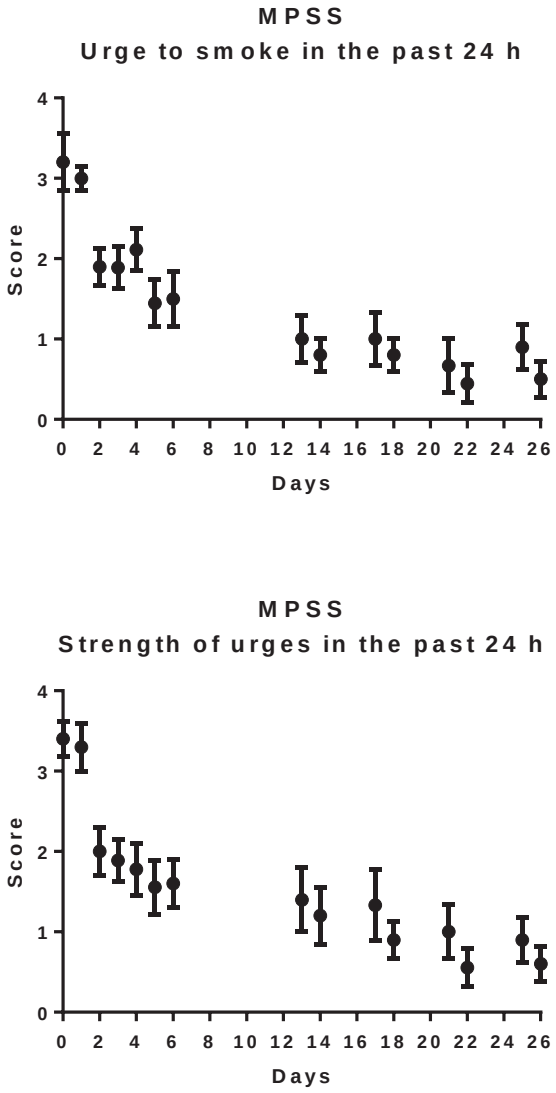

Figure 5.9: Urge to smoke in the past 24 hours (left panel) and strength of urges in the past 24 hours (right panel) measured using the MPSS in 10 participants. Values are shown as mean ± SEM.

Notes: Possible scores for urge to smoke are from 0 (not at all) to 5 (all the time). Possible scores for strength of urges are from 0 (no urges) to 5 (extremely strong).

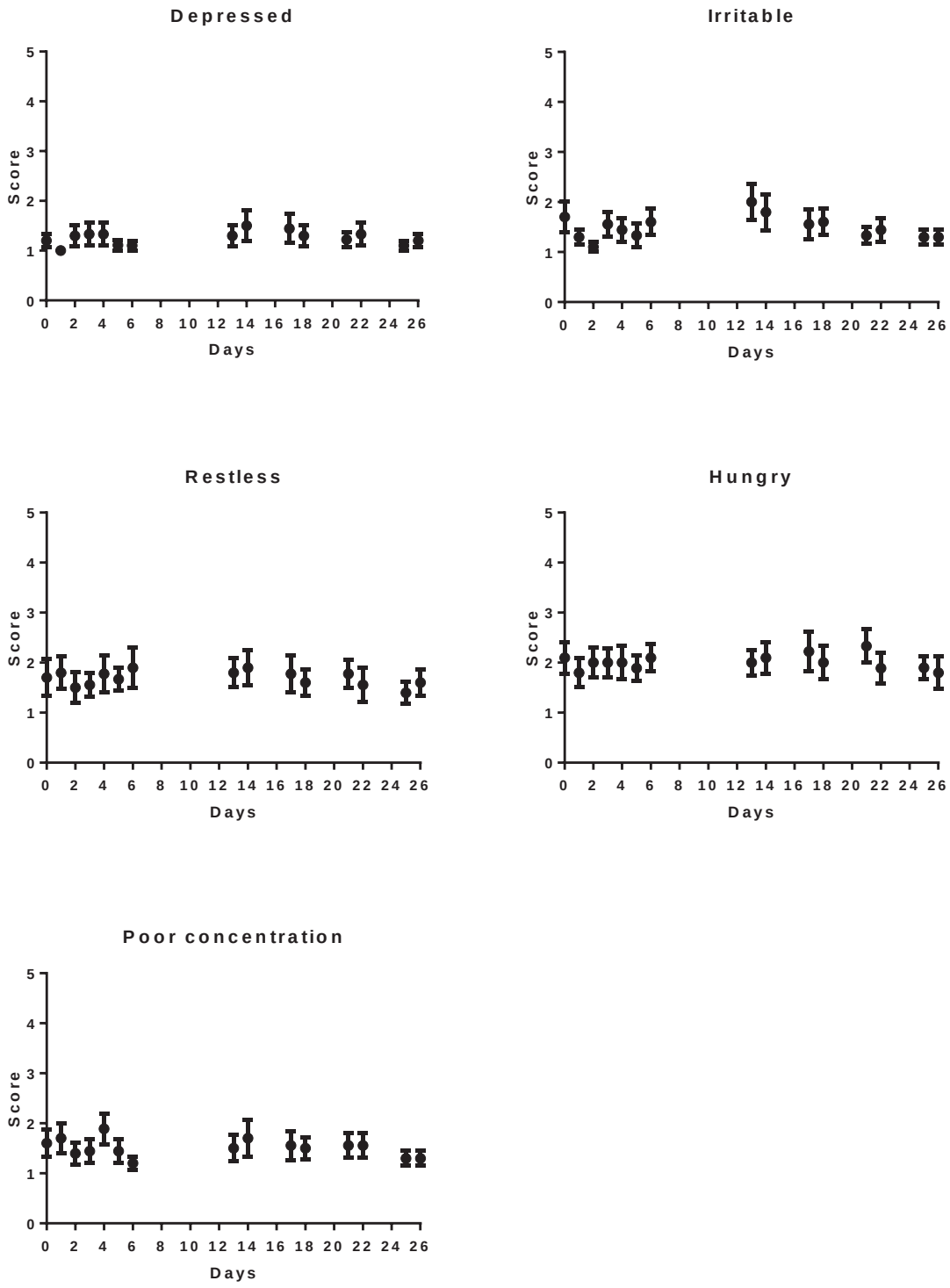

Figure 5.10: Severity of withdrawal symptoms measured using the MPSS in 10 participants during the study. Values are shown as mean ± SEM.

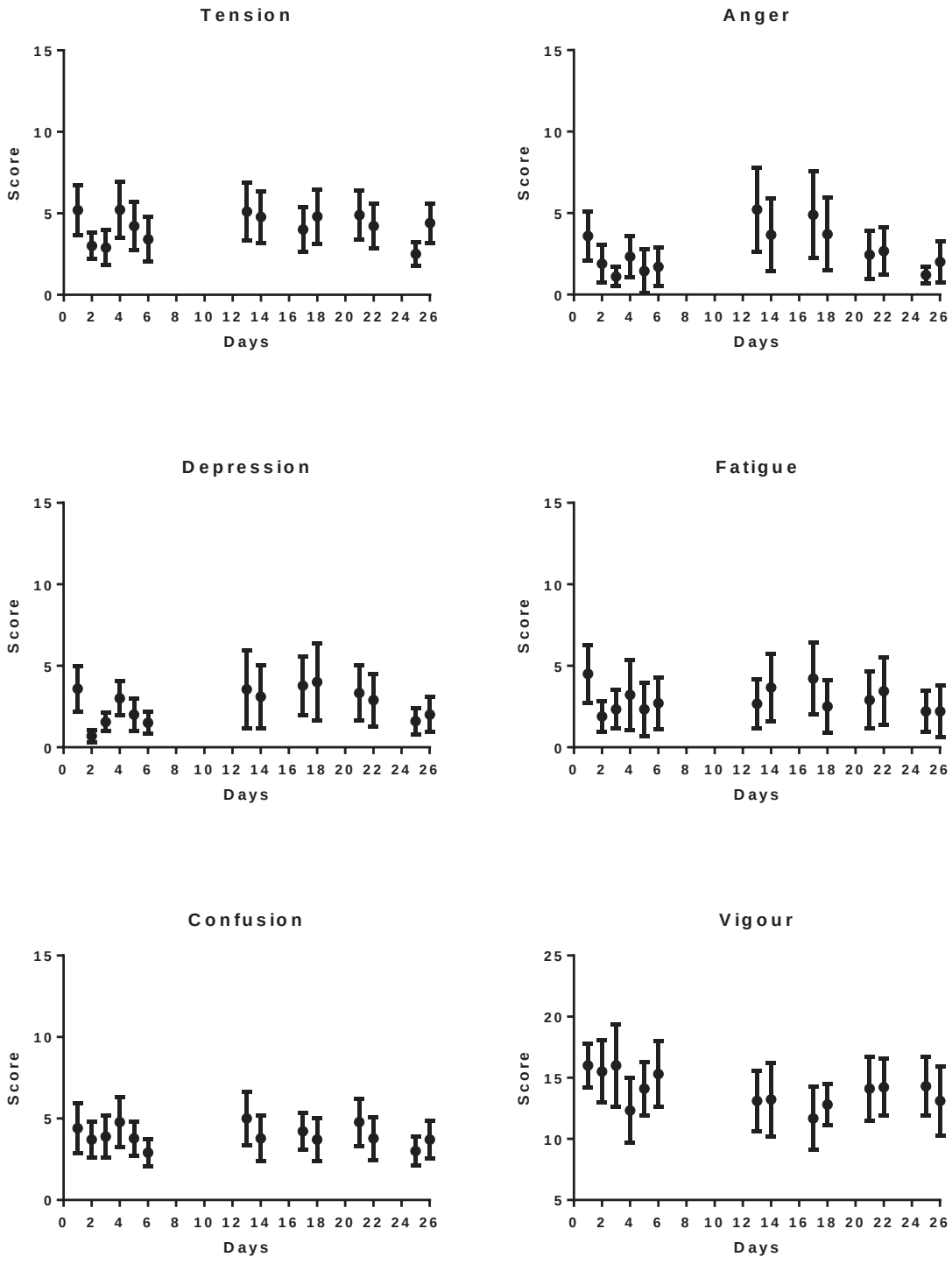

Figure 5.11: Subscales of mood measured using the POMS in 10 participants during the study. Values are shown as mean ± SEM.

Notes: A greater score indicates a greater mood disturbance except for vigour.

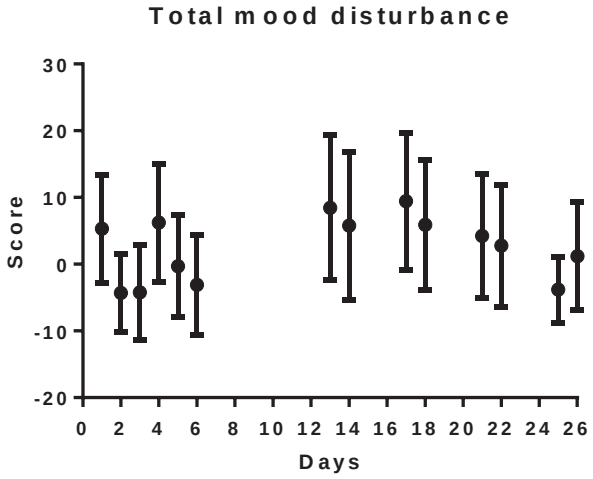

Figure 5.12: Total mood disturbance (TMD) scores measured using the POMS in 10 participants. Values are shown as mean ± SEM.

Notes: TMD is the sum of all negative mood subscales minus vigour. A greater score indicates a greater mood disturbance.

Table 5.6. Changes in cigarette craving, severity of withdrawal symptoms and mood in 10 participants between baseline (day 0) and day 26.

Item	Questionnaire	Possible scores	Baseline			Day 26			Z	p-value
			N	Mean score	95% CI	N	Mean score	95% CI		
Craving for cigarettes										
Craving score (at that time)	QSU-brief	10 - 70	10	39.7	[30.6 - 48.8]	10	11.9	[9.7 - 14.1]	-2.803	0.005*
Urge to smoke (in the past 24 h)	MPSS	0 - 5	10	3.2	[2.5 - 3.9]	10	0.5	[0.1 - 0.9]	-2.831	0.005*
Strength of urge (in the past 24 h)	MPSS	0 - 5	10	3.4	[3.0 - 3.8]	10	0.6	[0.2 - 1.0]	-2.844	0.004*
Withdrawal										
Depressed	MPSS	1 - 5	10	1.2	[0.9 - 1.5]	10	1.2	[0.9 - 1.5]	0.000	1.000
Irritable	MPSS	1 - 5	10	1.7	[1.1 - 2.3]	10	1.3	[1.0 - 1.6]	-1.134	0.257
Restless	MPSS	1 - 5	10	1.7	[1.0 - 2.4]	10	1.6	[1.1 - 2.1]	0.000	1.000
Hungry	MPSS	1 - 5	10	2.1	[1.5 - 2.7]	10	1.8	[1.2 - 2.4]	-0.722	0.470
Poorconcentration	MPSS	1 - 5	10	1.6	[1.1 - 2.1]	10	1.3	[1.0 - 1.6]	-1.732	0.083
Mood										
Tension	POMS	0 - 36	10	5.2	[2.2 - 8.2]	10	4.4	[2.0 - 6.8]	-0.724	0.469
Depression	POMS	0 - 60	10	3.6	[0.8 - 6.4]	10	2	[-0.1 - 4.1]	-1.691	0.091
Anger	POMS	0 - 48	10	3.6	[0.7 - 6.5]	10	2	[-0.5 - 4.5]	-2.032	0.042*
Fatigue	POMS	0 - 28	10	4.5	[1.0 - 8.0]	10	2.2	[-0.9 - 5.3]	-2.207	0.027*
Confusion	POMS	0 - 28	10	4.4	[1.4 - 7.4]	10	3.7	[1.4 - 6.0]	-1.186	0.236
Vigour	POMS	0 - 32	10	16	[12.5 - 19.5]	10	13.1	[7.5 - 18.7]	-1.246	0.213
Total mood disturbance	POMS	-32 - 200	10	5.3	[-10.6 -21.2]	10	1.2	[-14.7 - 17.1]	-1.326	0.185

Abbreviations: QSU-brief=brief Questionnaire on Smoking Urges; MPSS= Mood and Physical Symptoms Scale; POMS=Profile of Mood States.
Z value obtained from Wilcoxon Rank test (* indicates statistical significance ($p<0.05$)).

5.6.4 Smokers vs non-smokers

5.6.4.1 *Are craving scores higher in non-smokers than smokers?*

During the first 4 days of the study, when all participants were smoking cigarettes, the mean cigarette craving, as measured using the QSU-brief, decreased continually. On day 5, there was no apparent difference in the mean craving scores between participants who had quit smoking and participants who continued to smoke. Over the remainder of the study period, the mean craving scores appeared to decrease in participants who had quit smoking (Figure 5.13). On the other hand, mean craving scores in participants who continued to smoke appeared to be resistant to change (Figure 5.13). At the end of the study, the mean cigarette craving score in participants who had quit smoking was less than the mean craving score in participants who continued to smoke cigarettes. Similar results were shown for the urge to smoke and strength of urge items in the MPSS (Figure 5.14).

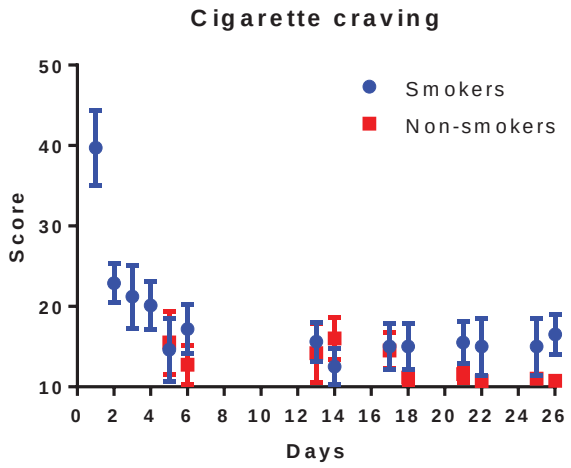

Figure 5.13: Cigarette craving scores measured using the QSU-brief in smokers and non-smokers. Values are shown as mean ± SEM.

Notes: The number of participants in each group changed throughout the study as participants quit smoking on different days.

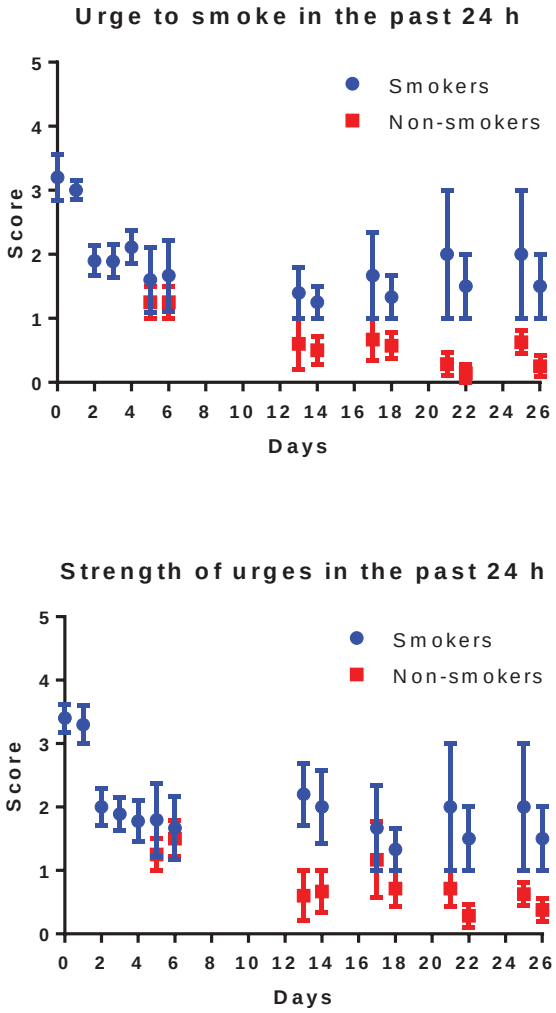

Figure 5.14: Urge to smoke in the past 24 hours (top panel) and strength of urges in the past 24 hours (bottom panel) measured using the MPSS in smokers and non-smokers.

Notes: Possible score for urge to smoke in the past 24 hours (top panel) is 0 (not at all) to 5 (all the time) and possible scores for strength of urges in the past 24 hours (bottom panel) is 0 (no urges) to 5 (extremely strong).

5.6.4.2 *Is there greater mood disturbance in non-smokers than smokers? (POMS)*

Overall, the mean total mood disturbance scores were greater in participants who had stopped smoking than in participants who continued to smoke (Figure 5.15).

5.6.4.3 *Are withdrawal symptoms more severe in non-smokers than smokers? (MPSS items)*

The mean scores for depression, irritability, restlessness, hunger and poor concentration were higher in participants who had stopped smoking than in participants who continued to smoke (Figure 5.16).

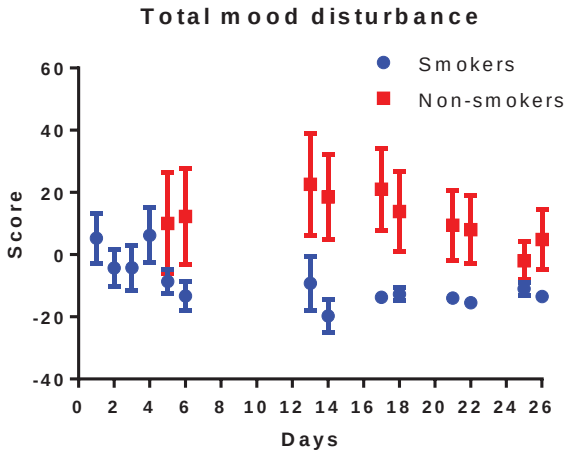

Figure 5.15: Total mood disturbance scores measured using the POMS in participants who were smoking (blue dots) and in participants who quit smoking (red squares). Values are shown as mean ± SEM.

Notes: TMD is the sum of all negative mood subscales minus vigour. A greater score indicates a greater mood disturbance.

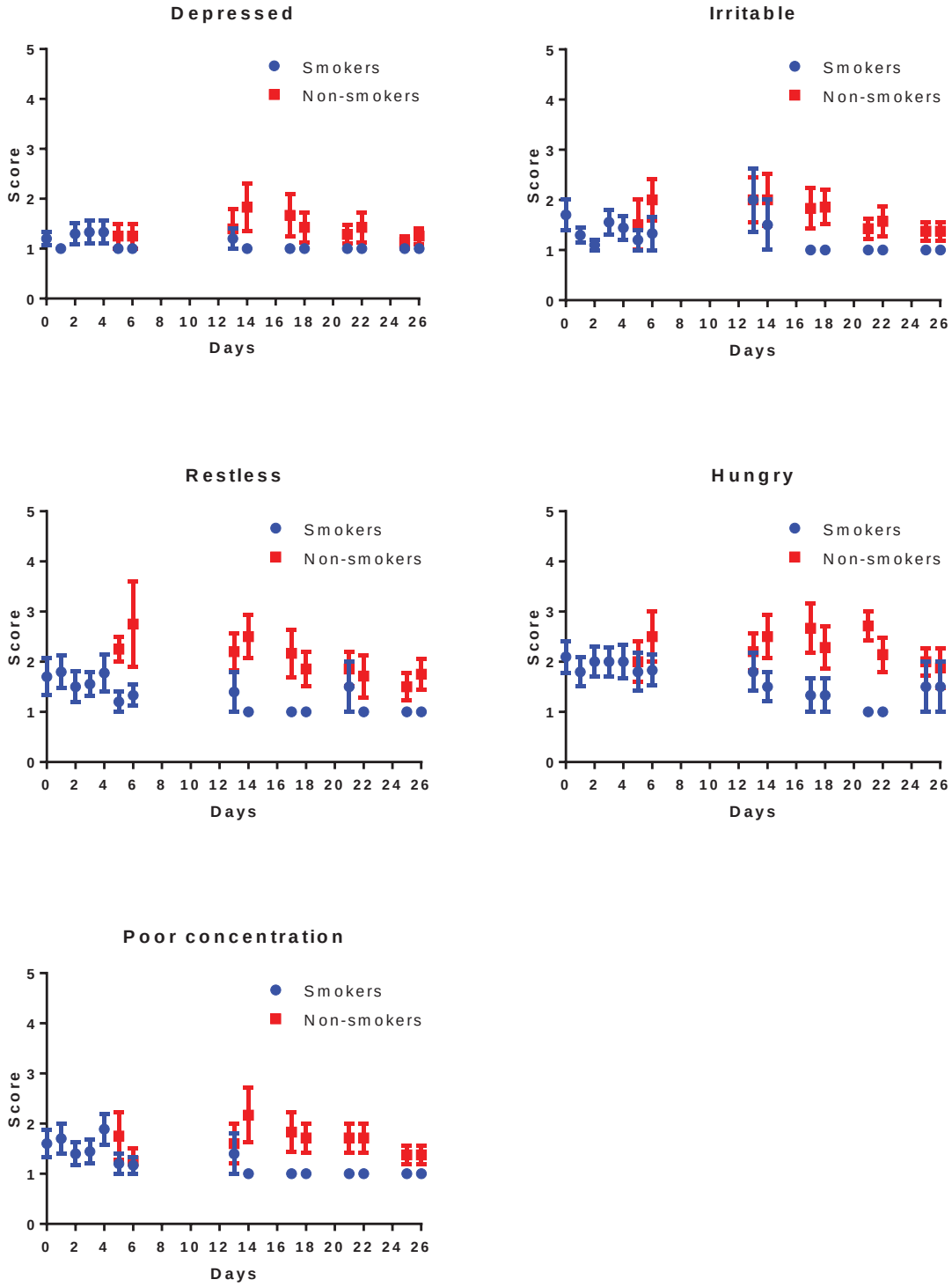

Figure 5.16: Severity of withdrawal symptoms measured using the MPSS in smokers and non-smokers. Values are shown as mean ± SEM.

5.6.5 Why did only some participants quit on Day 5 while others did not?

5.6.5.1 *Are there differences in craving in participants who quit on Day 5 and those who do not?*

There was between-subject variability in baseline (day 1, 0h) craving scores. No distinct differences or patterns were observed across days 1 to 6 in the individual craving scores between participants who had stopped smoking on day 5 and participants who had not stopped smoking on day 5 (Figure 5.17).

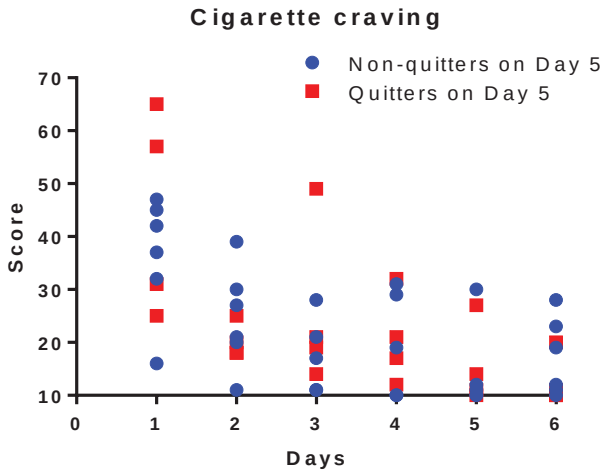

Figure 5.17: Cigarette craving scores measured using the QSU-brief on Days 1 – 6 in participants who did not quit smoking on Day 5 (blue dots) and in participants who quit smoking on Day 5 (red squares) .

5.6.5.2 *Are there differences in smoking satisfaction in participants who quit on Day 5 and those who do not?*

As for cigarette craving, no clear differences were observed for smoking satisfaction between participants who had stopped smoking on day 5 and participants who did not quit smoking on day 5 (Figure 5.18).

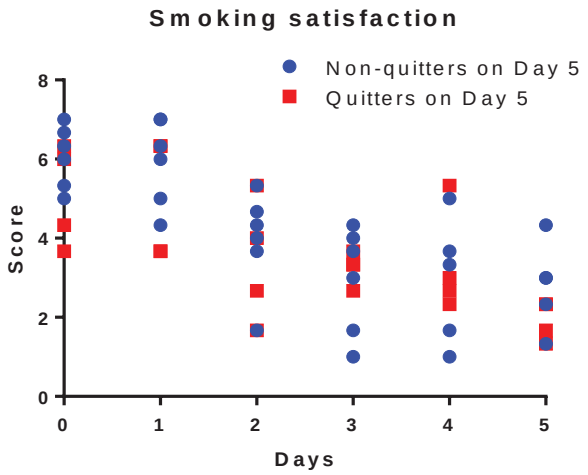

Figure 5.18: Smoking satisfaction scores measured using the mCEQ on Days 1 – 5.

5.6.6 Can we predict who will quit based on subjective measures?

Based on the results above, it was not possible to discriminate participants who stopped smoking on day 5 from participants who did not stop smoking on day 5, based on cigarette craving or smoking satisfaction. However, day 5 was not the only quit day for participants; there were participants who stopped smoking on different days of the study beyond day 5. This led to the question of whether it would be possible to predict who was going to have stopped smoking in the study (regardless of the quit day).

Another way to answer this question is to determine whether it was possible to predict the participants who did not stop smoking. In other words, were the 2 participants who did not stop smoking in the study different from those who did stop smoking?

Craving scores for the two participants (participant 6 and 9) who were smoking at the end of the study were compared to the mean craving scores for the eight participants who had quit smoking. As with any other participant in the study, craving scores for participant 6 and participant 9 had decreased during the study. At the end of the study, craving scores in both participants were reduced by more than 2-fold compared to their baseline scores. An interesting feature in participant 6 was that the craving score continuously decreased for the first few days of the study and on day 4 this participant reported to have “no cravings” (indicated by a score of 10, the lowest possible score on the QSU-brief). However, this pattern did not continue from day 6 onwards. The craving score

increased on day 4 and appeared to be resistant to change until the end of the study. Participant 6 had a higher craving score at the end of the study than any other participant in the study. On the other hand, craving scores for participant 9 continuously decreased in the study. At the end of the study, participant 9 had very little cigarette craving, similar to the participants who had quit smoking in the study (Figure 5.19).

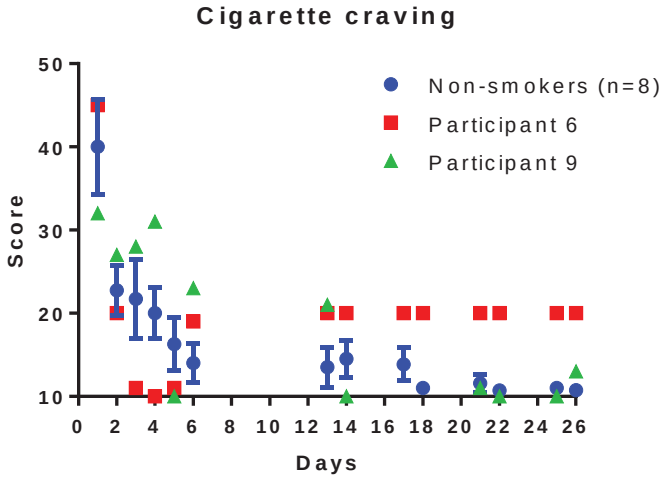

Figure 5.19: Mean ± SEM cigarette craving scores measured using the QSU-brief in participants who quit smoking (n=8) in the study and individual craving scores for 2 participants (Participant 6 and 9) who did not quit smoking.

Results from the mCEQ for these two participants showed that the participants felt that smoking was less satisfying at the end of the study than at the start of the study (Figure 5.20). On day 26, the smoking satisfaction ratings in these participants were between “very little” to “a little” compared to “extremely” or “quite a lot” at baseline. Similar to this, the two participants also felt that smoking was less psychologically rewarding at the end of the study than at the start of the study (Figure 5.21). Enjoyment of respiratory tract sensations had decreased for both participants in the study. At the end of the study, both participants felt that sensations in the throat and chest from smoking were “very little” or “not at all” enjoyable (Figure 5.22).

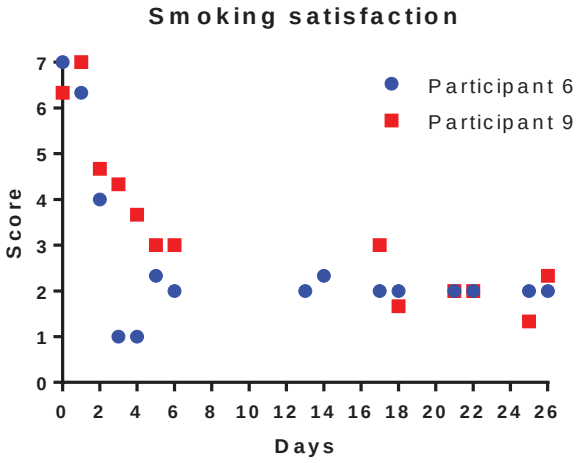

Figure 5.20: Smoking satisfaction measured using the mCEQ in 2 participants who did not quit smoking.

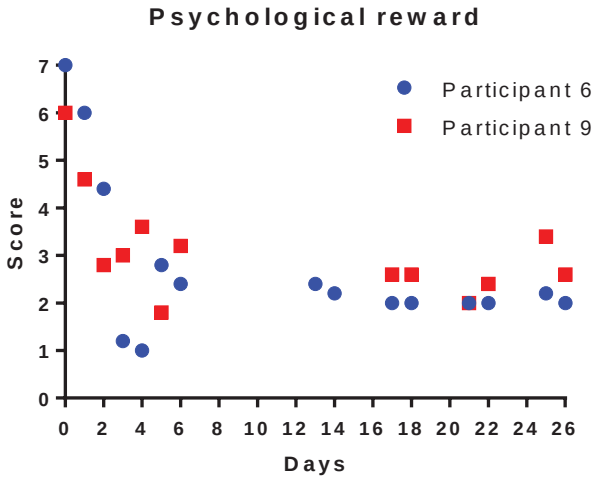

Figure 5.21: Psychological reward measured using the mCEQ in 2 participants who did not quit smoking.

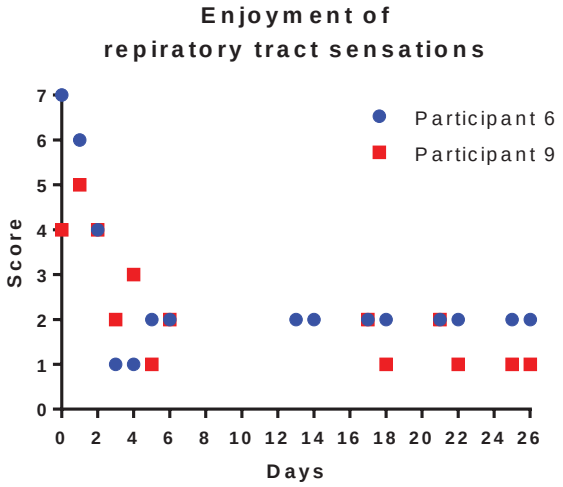

Figure 5.22: Enjoyment of respiratory tract sensations measured using the mCEQ in 2 participants who did not quit smoking.

From these results, it was not possible to predict (retrospectively) that the two participants would not quit smoking. In other words, no clear differences were observed in the cigarette craving between participants who quit smoking and those who did not quit smoking. In addition, there was a decrease in smoking satisfaction, psychological reward and enjoyment of respiratory tract sensations in these two participants, but this did not lead them to quit cigarette smoking.

5.7 Part C: Patterns of smoking

5.7.1 Overview

Results of the smoking section of the study diary are presented in this section. This section will present the changes in the number of cigarettes smoked per day for each participant and the changes in smoking patterns. The changes in mean number of cigarettes smoked will be compared to the changes in craving scores

5.7.2 What happens to cigarette smoking during the study?

All participants reported smoking fewer cigarettes per day at the end of the study compared with baseline (Figure 5.23 and Figure 5.24). Five participants reported stopping smoking on day 5

(participants 1, 2, 4, 5 and 7). Participants 1, 2 and 4 gradually reduced the number of cigarettes from day 1 to day 4. This pattern was not observed for the other participants (participant 5 and 7). Of these participants, four did not relapse during the study. Participant 7 stopped smoking from day 5 to day 9, but reported to take "puffs" or smoke "half" a cigarette on days 10, 13 and 16.

Participant 6 reported stopping smoking on day 3, but took "puffs" (e.g. 2 puffs) of cigarette smoke on days 4 – 8, 13 and 20 – 25. Participant 9 reported stopping smoking on days 6 – 8, but relapsed and continued to smoke throughout the remaining study period. In the study diary, this participant reported that they had experienced a very stressful situation on day 7 and this made them go back to smoking. However, this participant reported smoking fewer cigarettes per day than at baseline.

Participant 10 reported stopping smoking on day 6. On day 13, this participant reported taking "puffs" of cigarettes as they were "tempted" at a concert, but did not smoke any more cigarettes throughout the study.

Looking at the patterns of cigarette smoking in the 4 participants who quit smoking on day 5 (Participant 1, 2, 4 and 5), except for one participant (Participant 5), the number of cigarettes smoked per day decreased throughout the days prior to quitting (Days 1 – 4) (Figure 5.25).

Time taken to smoke the first cigarette after waking up is an important indicator of the severity of cigarette dependence, an item in the FTND (Heatherton, Kozlowski et al. 1991) and, therefore, was explored for days when participants were all smoking (Days 1 – 4). The number of participants who smoked within 5 minutes after waking up declined after day 1. The number of participants who smoked their first cigarette between 6 and 30 minutes from waking up increased from day 1 to day 2, but decreased on days 3 and 4. Fewer participants smoked their first cigarette between 6 and 30 minutes on day 4 than on day 1. On the other hand, participants who smoked their first cigarette > 1 hour from waking up had increased from day 1 to day 4. This indicated that the mean time taken to smoke the first cigarette after waking up had increased from day 1 to day 4.

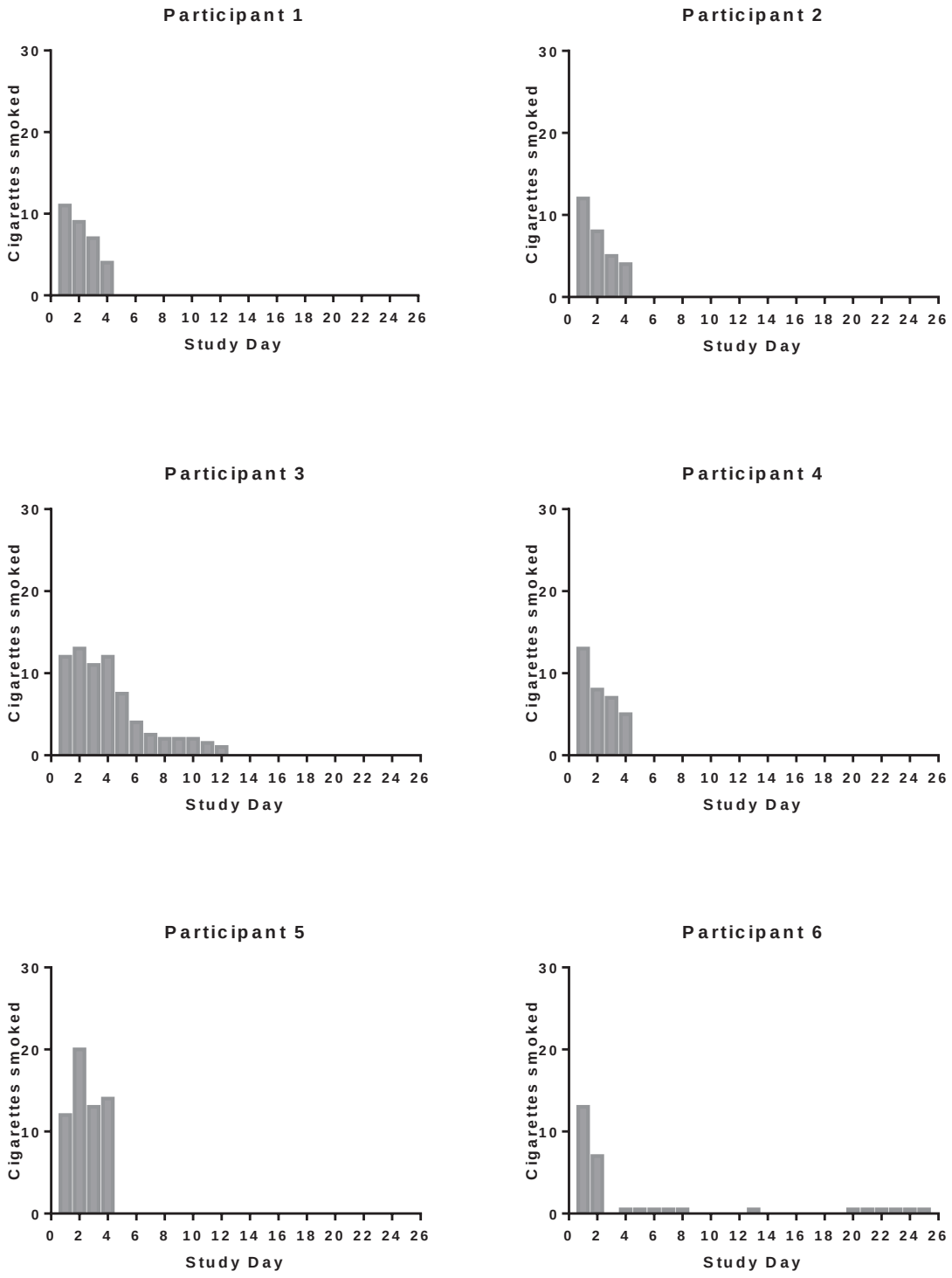

Figure 5.23. Self-reporting of number of cigarettes smoked per day for participants 1 – 6.

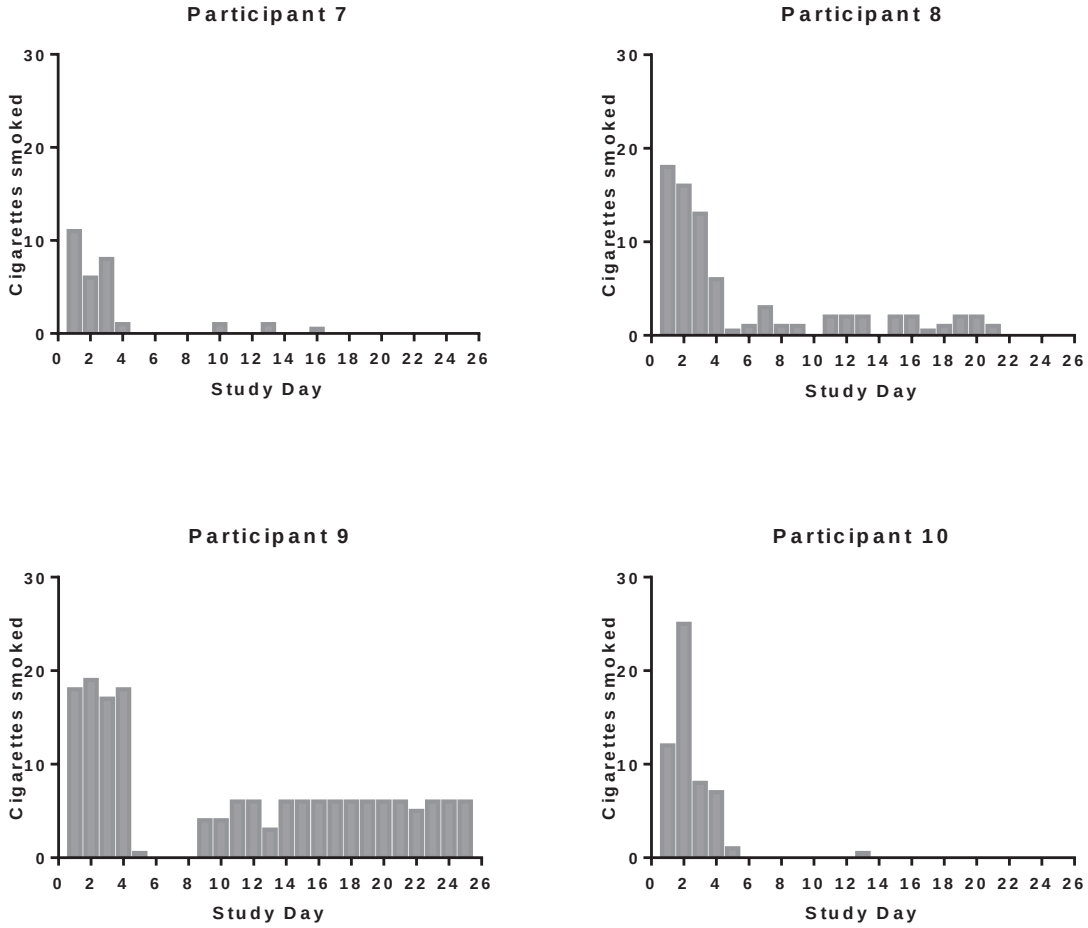

Figure 5.24. Self-reporting of number of cigarettes smoked per day for participants 7 – 10.

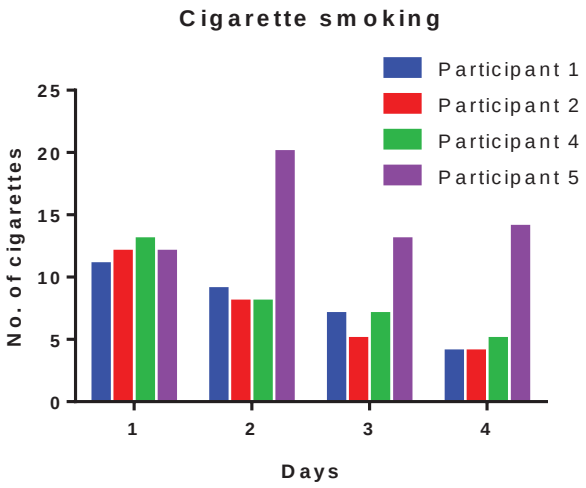

Figure 5.25: Cigarettes smoked per day on Days 1 – 4 in participants (participants 1, 2, 4 and 5) who quit smoking on Day 5.

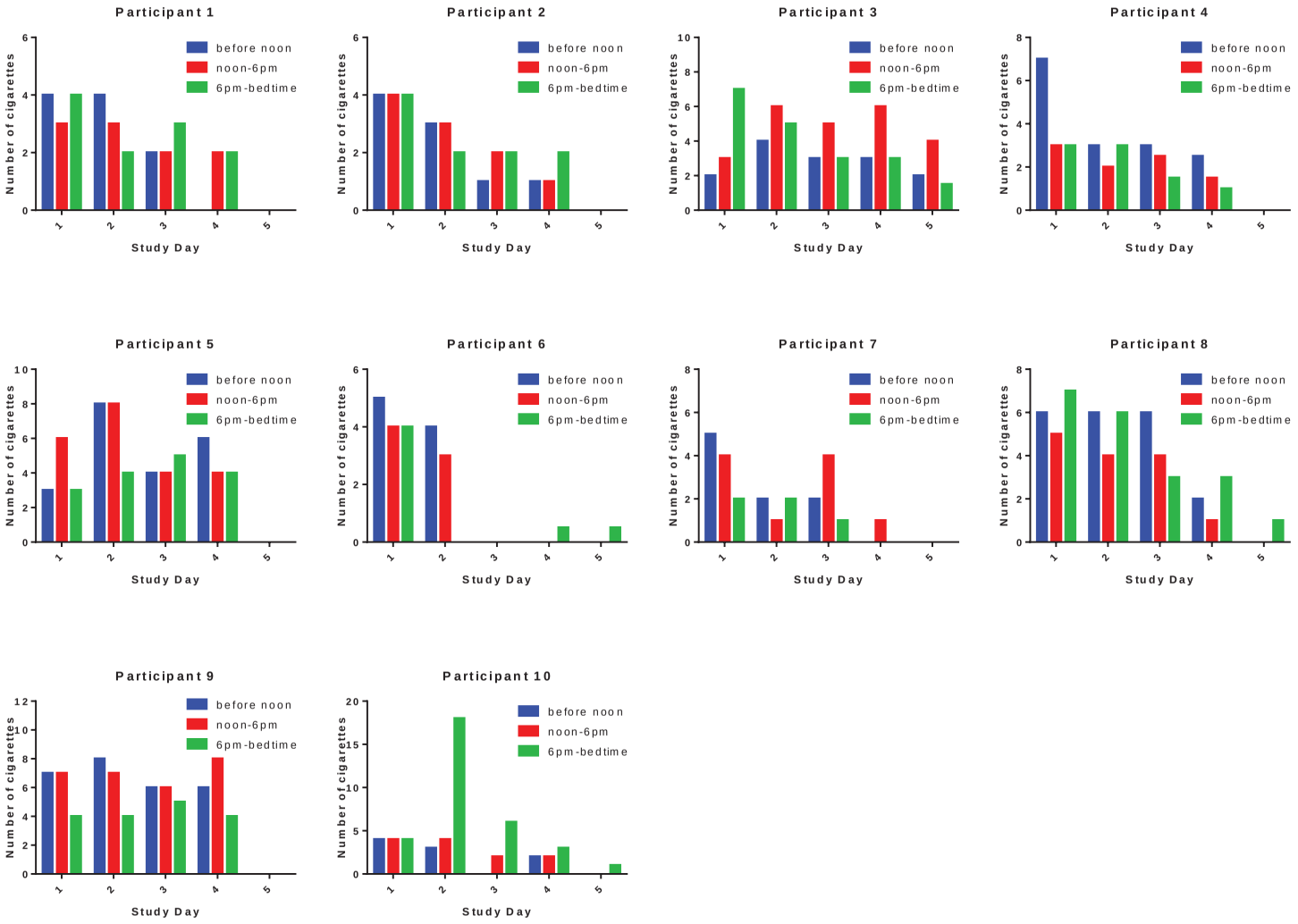

Figure 5.26. Patterns of smoking during days 1 – 5.

5.7.3 Is smoking related to craving?

On day 1, the mean ± SD number of cigarettes smoked per day was 13.2 ± 2.6 in 10 participants (self-report). At the end of the study, the mean number of cigarettes smoked per day was 0.7 ± 2.0. At the end of the study, participants had either quit smoking or smoked fewer cigarettes per day. Decreases in the number of cigarettes smoked appeared to coincide with decreases in mean cigarette craving as measured using QSU-brief throughout the study (Figure 5.27).

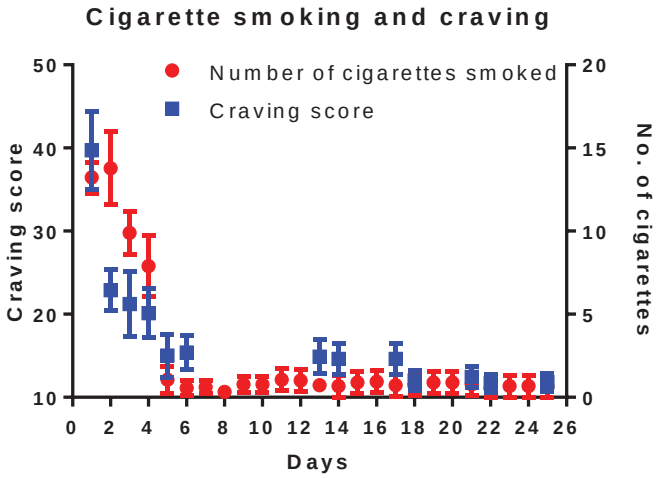

Figure 5.27: Changes in number of cigarettes smoked per day and craving scores in 10 participants who were following the recommended dosing regimen of Tabex®. Values are shown as mean ± SEM.

Notes: Cigarettes smoked per day was self-reported in the study diary. Craving scores were measured using QSU-brief.

5.8 Part D: Concentration-effect relationships

5.8.1 Overview

This section attempts to link the findings from Part A to the findings in Part B in an effort to investigate whether the subjective effects measured in smokers can be related to plasma cytisine concentration. Based on results presented up to now, no significant changes were observed in heart rate, blood pressure and respiratory rate even on day 1 when there was frequent administration of cytisine. Also, no changes were observed with withdrawal symptom severity. On the other hand, changes in cigarette craving were observed. Therefore, an investigation was conducted to explore

whether cigarette cravings were related to plasma cytisine concentration. It was also of interest to explore whether plasma cytisine concentration was associated with quitting.

5.8.2 Is there a relationship between plasma cytisine concentration and cigarette craving?

During the first day of the study, when repeated dosing led to increases in cytisine plasma concentrations, it was also found that there was a decrease in the mean craving scores (measured using QSU-brief) (Figure 5.28). This suggests that there may be an inverse relationship between cytisine concentration and craving for cigarettes. However, changes in craving scores (baseline corrected) were not highly correlated with cytisine concentrations measured in plasma over the duration of the study (R^2=0.2409): A higher plasma concentration of cytisine did not always correspond to a greater reduction in craving scores (Figure 5.29).

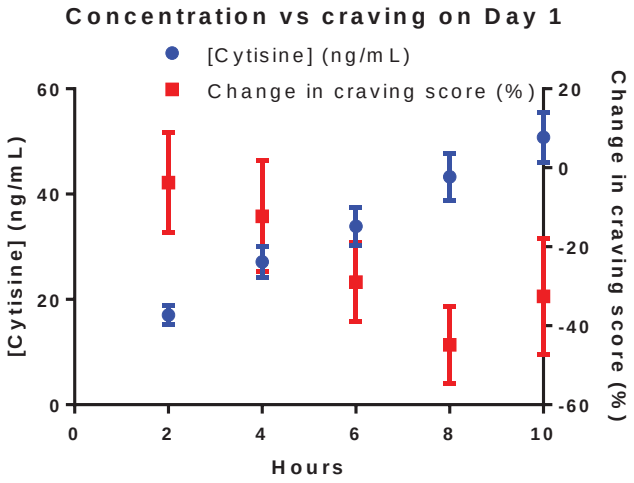

Figure 5.28: Plasma trough concentration-time profile and the changes in craving scores (QSU-brief) from baseline on Day 1. Values are shown as mean ± SEM (n=10).

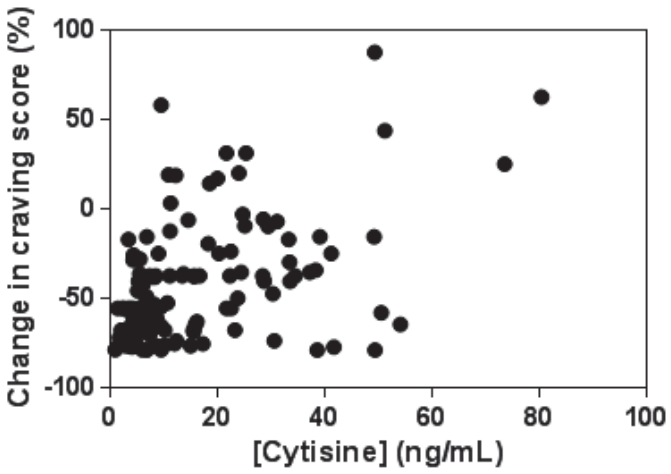

Figure 5.29: Individual cytisine plasma concentration vs. change in craving score (QSU-brief).

5.8.3 Is there a relationship between plasma cytisine concentration and quitting?

There was no apparent relationship between plasma cytisine concentration and quitting smoking. Of the two participants who continued to smoke, only one of them had lower cytisine plasma concentrations compared to other participants in the study. The participant who had the highest cytisine concentration on day 1 was not one of the first participants to quit smoking.

5.9 Part E Validation of smoking cessation

5.9.1 Overview

Although this was not a smoking cessation trial, smoking cessation was confirmed with cotinine biochemical validation.

5.9.2 Self-reported smoking vs. cotinine validation

Not all participants stopped smoking in the study, and as shown in the Kaplan-Meier survival curve (Figure 5.30), participants reported quitting smoking at different times during the study. As the study progressed, biochemical validation showed that NicAlert® (cotinine) levels decreased from baseline for all participants (Figure 5.31 and Figure 5.32). This was consistent with self-reported smoking

(Figure 5.23 and Figure 5.24). Overall, the decreasing trend in the craving scores (measured using QSU-brief) appeared to be consistent with the decreasing trend in smoking confirmed by cotinine levels (Figure 5.33). At the end of the study, 8 of the 10 participants were biochemically verified to have quit smoking (*cf*. 9 self-reported quitting).

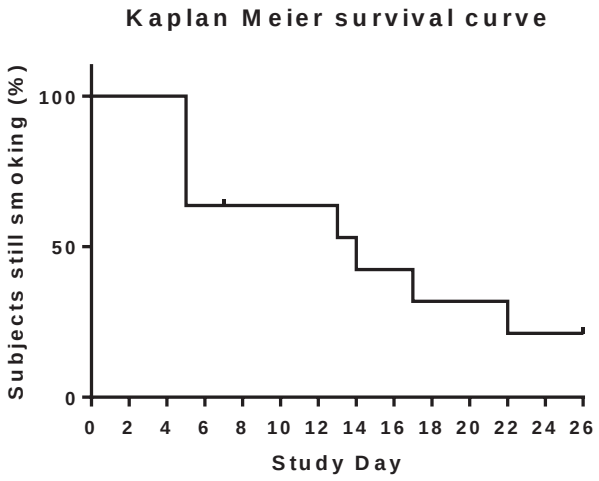

Figure 5.30: Kaplan-Meier survival curve showing the percentage of smokers in the study.

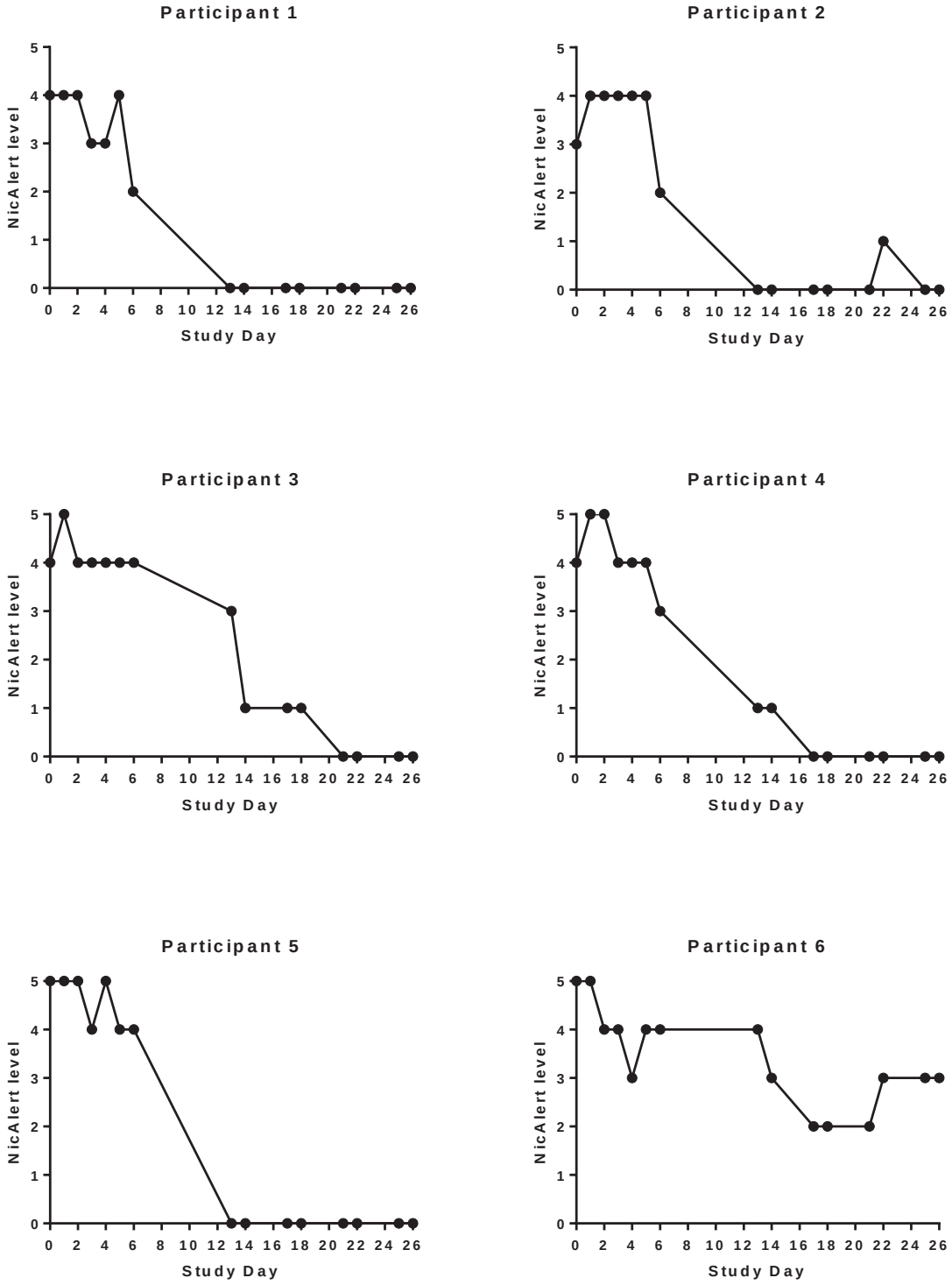

Figure 5.31. NicAlert® levels (cotinine) in participants 1 – 6 from baseline (screening) to Day 26.

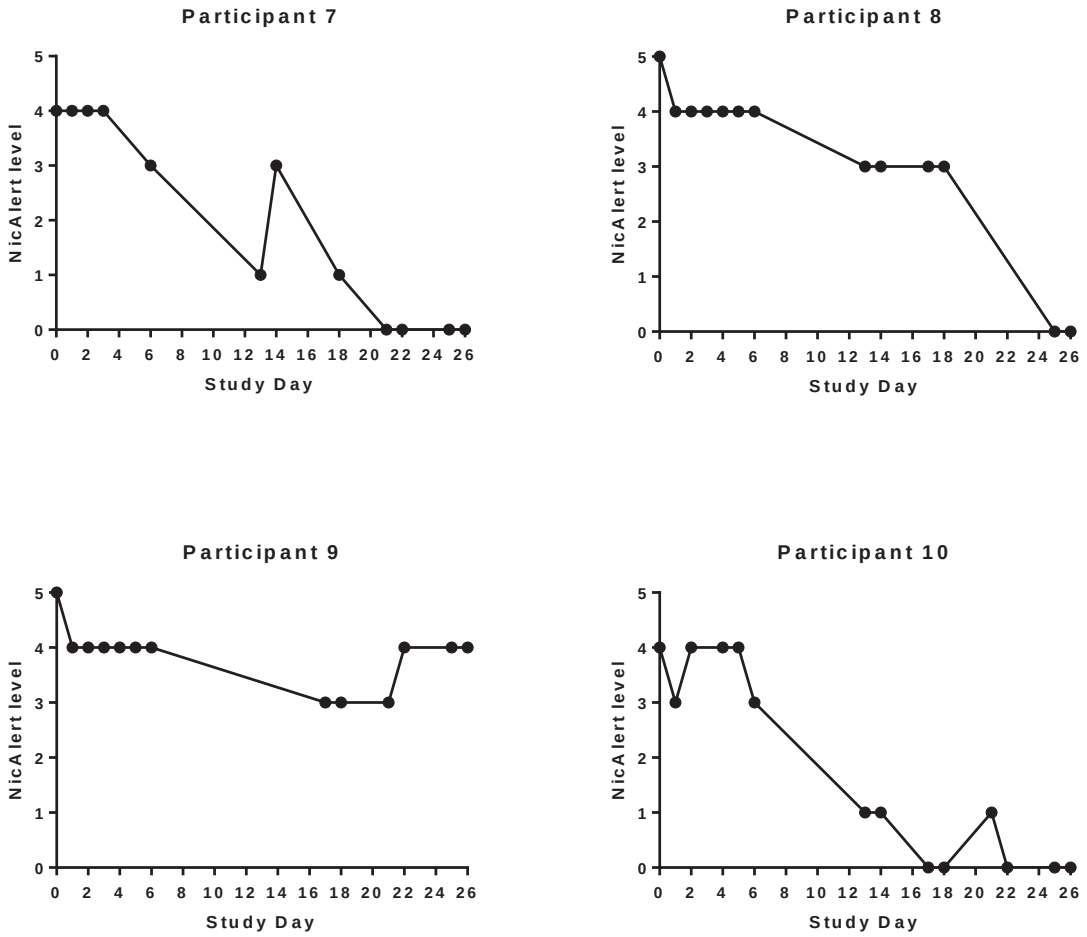

Figure 5.32. NicAlert levels (cotinine) measured in participants 6 – 10 from baseline (screening) to Day 26.

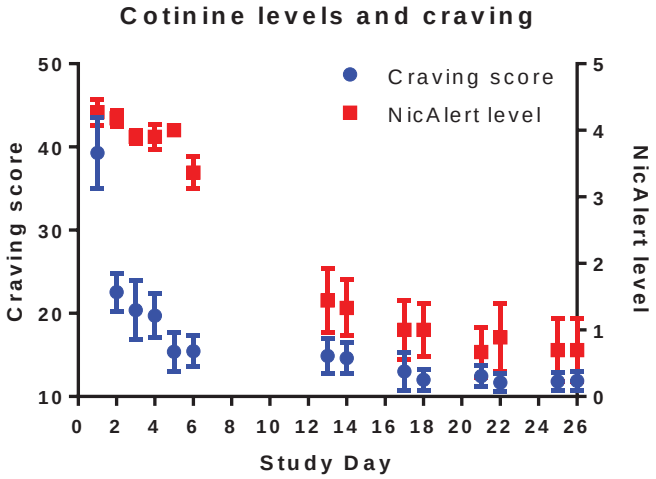

Figure 5.33: Cotinine levels as measured using NicAlert® and cigarette craving (QSU-brief) over the study period. Values are shown as mean ± SEM (n=10).

5.10 PART F: Safety and medication adherence

5.10.1 Overview

This section will present the self-reported side effects and results from the sleep section of the study diary. In addition, a description of the - participant who withdrew from the study is provided. Results from the dosing section of the study diary and tablet count will also be presented.

5.10.2 Self-reported side effects

The most commonly reported side effects included changes in appetite, dry mouth, cigarettes tasting less pleasant, dreams/nightmare and irritability. No patterns were observed with these side effects. A detailed list of self-reported side effects and their frequency throughout the study is presented in Table 5.7.

5.10.2.1 *Sleep*

Self-reported mean ± SD number of total hours slept each day at the start of the study was 6.4 ± 2.0 hours. Although there were fluctuations in the mean number of sleep during the study and ratings on quality of sleep, overall, cytisine did not appear to have affect sleep (no major sleep disturbances)

in participants following the recommended dosing regimen of Tabex® (Figure 5.34 and Figure 5.35). Four out of ten participants experienced dreams/nightmares and this was one of the most commonly reported side effects in the participants. Two participants reported that they had experienced sleep problems during the study (insomnia or difficulty sleeping).

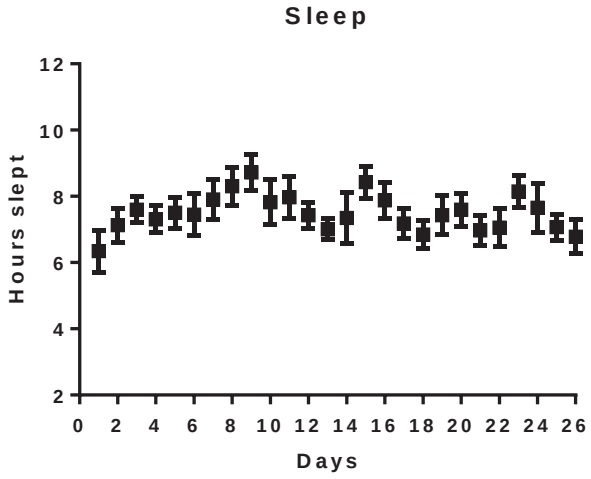

Figure 5.34: Hours slept per day in participants following the recommended dosing regimen of Tabex®. Values are shown as mean ± SEM (n=10).

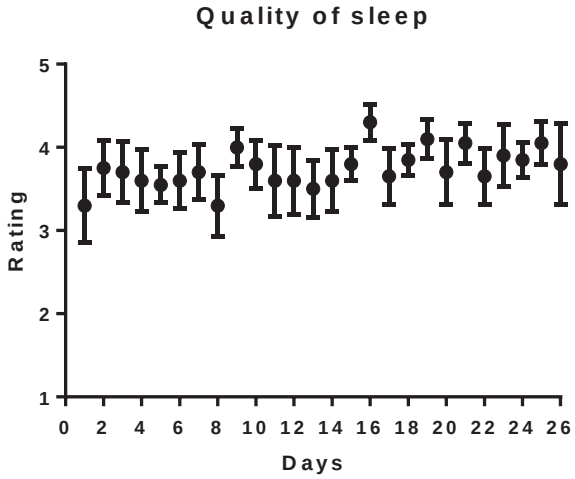

Figure 5.35: Ratings on sleep quality in participants while following the recommended dosing regimen of Tabex®. Values are shown as mean ± SEM (n=10).

Notes: The quality of sleep was self-reported in the study diary. Scale is from 1 (very restless) to 5 (very sound).

5.10.2.2 *Participant who withdrew from the study*

Two unusual occurrences were reported by the participant who withdrew from the study. These included reduced libido and abdominal pain. Reduced libido was reported on day 7 and lower abdominal pain was reported on day 10 in the study diary. The reason for withdrawing from the study was due to abdominal pain (self-report).The participant reported that they had felt moderate pain in the lower abdominal area and noticed a change in urine colour. The participant concluded that the pain may have been due to a kidney stone as they had them in the past, but the pain was thought to be unusual because there was no back pain. The participant took tramadol for pain relief and stopped taking cytisine. The participant reported that the pain disappeared the next day and noticed that their urine colour came back to normal. The participant did not seek any medical advice before their next planned visit. The participant did not continue taking cytisine even though the pain had been alleviated. The participant had a brief session (approximately 30 minutes) with the Medical Officer at this next clinic visit. The Medical Officer recorded the details of the adverse event in the Adverse Events Form (Appendix 8).

There were no noticeable differences in mood and withdrawal during the time the participant took part compared to the rest of the participants.

However, an interesting finding was that this participant had extreme difficulty in adhering to the dosing regimen as recorded in the dosing diary. This participant frequently forgot to take a tablet at the correct time and, despite being told not to, reported taking two tablets at the one time to make up for the missed dose.

5.10.3 Medication adherence

Of the 10 participants who completed the study, only one participant recorded having fully adhered to the recommended dosing regimen. Most participants either forgot to take a tablet or took tablets at incorrect dosing intervals at some point during the study. Seven participants reported missing at least one dose during the study. From the dosing diary, it was evident that participants found it difficult to remember the dosing interval because it frequently changed throughout the course of the treatment. In one report, a participant got confused with the study day and therefore took the wrong number of tablets at wrong dosing intervals. Overall, the reports from the self-reported dosing diary coincided with the tablet counts at each visit.

Table 5.7. List of self-reported side effects and number of reporting each day during the study.

Symptoms	Days																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Total
Dry mouth/thirst	3	3	3		2		1		1		1	2	1			1	1		1			1					21
Constipated	1	1			1		1				1	2	1	1	1	1	2		1	1	2						17
Irritable/agitated/impatient/edgy/ mind wired/tense/restless/cranky		1	1	1	1			1		3	1	2		1		2	1	1	1								17
Appetite changes	1	4	1		1	1	1								1	2				1			2	1			16
Dreams/nightmare		1	1			2	1	1	1	1	1				1						1					1	12
Sore throat				1					1			1	1			1					2	2	1		1		11
Sleep disturbances			1	1	1		1			1	1	1	1			2											10
Change in cigarette taste	3	1	1				1	1					1							1							9
Slow/sluggish/lethargy/drained/ no energy	1				1	1		1					1		1	1			1								8
Nausea			1					1	1		1					1	1										6
Sweating							1	1		1			1							1	1						6
Angry/bad mood/moody/annoyed						1	1			1		2															5
Coughing										1	1	1	1							1							5
Weight change						1		1	1				1												1		5
Bitter taste												1				1									1	1	4
Dizzy																				4							4
Gloomy/emotional/blue/down/low			1					1		1				1													
Headache				1							1								1		1						4
Abdominal pain												1	2														3
Runny nose/blocked nose				1							1		1														3
Diarrhoea		1																			1						2
Feeling sick													1	1													2
Uninterested/unmotivated												1					1										2
Anxious				1																							1
Chest pain									1																		1
Dry skin			1																								1
Flu like symptoms																						1					1
Hair loss																					1						1
Lung pain													1														1
Mental state not great													1														1
Reduced libido				1																							1
Urine colour/smell change																				1							1
White coating on tongue																1											1

Notes: “1” indicates the occurrence of the adverse event. All adverse events were self-reported in the study diary.

5.11 Discussion

This chapter reports the findings from a multi-dose study of cytisine in 10 participants who were instructed to follow the standard dosing regimen of Tabex®. Pharmacokinetics of cytisine and its impact on physiological (heart rate, blood pressure and respiratory rate) and psychological (cigarette craving, withdrawal, mood and smoking satisfaction) effects have been presented. From these data, the relationship between these effects (in particular craving for cigarettes) and plasma concentrations of cytisine was explored.

In the previous chapter, the pharmacokinetics of cytisine following a single dose was described. The mean half-life of cytisine was found to be approximately 4.8 hours in relatively fit and young male smokers (Chapter 4)(Jeong, Newcombe et al. 2014). The time to reach a steady state concentration would therefore be estimated to be around 20 hours (after 4 half-lives). The initial recommended dosing (the most intense dosing period) however is 1.5 mg every 2 hours for a maximum of 6 times per day (Sopharma 2008). This means that the last tablet (6^{th} dose) taken during the first day would be approximately 10 hours after the first dose. Thus, it would be predicted that a steady state concentration would not be reached under normal dosing conditions. The estimated mean elimination half-life of cytisine (4.9 hours) was thus very similar to that obtained following a single dose administration.

It was hypothesised that multiple dosing would lead to accumulation of cytisine in plasma. Based on the half-life determined in Chapter 4, it was expected that the current Tabex® dosing regimen would lead to accumulation of cytisine in plasma but steady state concentrations would not be maintained during the course of the therapy. It was also hypothesised that there would be a relationship between plasma cytisine concentration and psychological effects associated with smoking.

Following multiple doses on day 1, accumulation of cytisine was observed in plasma for all participants. However, there was minimal day-to-day accumulation of cytisine in plasma. This was because of the large time difference between the last dose taken on a particular day and the first dose taken the next morning. For example, on day 1, participants took the first dose of cytisine at 8 am in the morning and took the last tablet (6^{th} dose) 10 hours later (6 o'clock in the evening). If participants then took the next tablet at 8 am the next morning (day 2), the time difference between the last dose of the first day (6^{th} dose) and the first tablet taken on day 2 (7^{th} dose) would be more than 12 hours. During this time, the concentration of cytisine would have continuously declined in plasma (elimination half-life of 4.9 hours). As a result, when the participants returned on day 2, cytisine trough plasma concentration had dropped to concentrations lower than the 2 hour sample

taken after the first dose. As the intensity of dosing decreased (dose tapering throughout the course of the treatment), there was a decrease in the plasma concentration of cytisine. Thus, the results were consistent with the hypothesis that cytisine does accumulate in plasma but steady state is not maintained with Tabex® dosing regimen.

In contrast to the situation with cytisine described above, varenicline has been shown to reach a steady state concentration under its recommended dosing regimen (Ravva, Gastonguay et al. 2009, Ebbert, Wyatt et al. 2010). This is expected as the half-life of varenicline (approximately 24 hours) is much longer than cytisine and it has been found to reach a steady state concentration within 4 days of treatment (Faessel, Gibbs et al. 2006). From a pharmacokinetic perspective, this is a major difference between cytisine and varenicline.

A striking difference between the findings from the single dose study and the multi-dose study was the variability observed in the plasma concentrations. In contrast to the single dose study, this study showed greater between-subject variability in cytisine concentrations. Variability in plasma cytisine concentrations was high even on day 1 where it was found that the differences in concentrations were more than 2 – 3-fold between participants. Pharmacokinetic modelling showed that this variability in cytisine concentration would be predicted throughout the study. Interestingly, it was possible to predict, by comparing cytisine concentrations measured on day 1, which participants would have higher concentrations of cytisine throughout the dosing period because participants with higher plasma cytisine concentrations on day 1 consistently showed higher cytisine concentrations throughout the whole duration of the treatment. It may, therefore, be important to explore between-participant variability in the pharmacokinetics of cytisine in a wider population of smokers in future studies.

Craving is transient (McBride, Barrett et al. 2006), therefore, it would be extremely difficult to capture the changes in craving during the early phase of the treatment if participants were asked to rate their craving once a day. To overcome this problem, this study used two different ratings for cigarette craving: the MPSS which allowed cravings to be looked at in the past 24 hour period (retrospective) and the QSU-brief to measure the "current" craving state. On day 1, intensive monitoring assessment of craving was done using the QSU-brief. This allowed a comprehensive profile of the early patterns of craving changes to be captured on day 1. This was one of the strengths of the study because craving can vary during the same day (Schneider and Jarvik 1983, Glassman, Jackson et al. 1984) but sometimes collected infrequently in many large trials to capture short-term trends.

The MPPS was also used to measure the participants' craving in the past 24 hours and may have been more appropriate in assessing craving during the rest of the study visits when participants were making short visits to the clinics. Regardless of the questionnaire used, the results showed that craving had decreased by the end of the study from baseline. However, this reduction in craving was not necessarily associated with quitting because the two participants who continued to smoke cigarettes also reported that they experienced low levels of cravings for cigarettes at the end of the study than at baseline.

The severity of withdrawal symptoms, excluding cigarette craving, did not change significantly at the end of the study compared with baseline. This is interesting because one would expect that withdrawal symptoms would worsen after a person reduces or completely stops smoking cigarettes (Hughes and Hatsukami 1986, Teneggi, Tiffany et al. 2002). This suggests that cytisine may be useful in relieving withdrawal symptoms during abstinence.

Mood was examined in this study since there have been ongoing concerns regarding depressive mood and behaviour disturbances in patients receiving varenicline (Stapleton 2009, US FDA 2009). Although the MPSS contains some overlapping mood items, the POMS (McNair, Lorr et al. 1971) was also used because it is a validated measure of mood extensively used in the literature and can provide a more comprehensive mood profile (Wewers, Tejwani et al. 1994, Wetter, Fiore et al. 1999). In this study, no serious concerns were identified and the total mood disturbance score did not change significantly over the duration of the study.

One of the questions posed in this study was whether smoking satisfaction and psychological reward were affected by cytisine therapy. A full analysis was not possible because participants who had quit smoking were no longer able to evaluate cigarette smoking. However, during the first 5 days of treatment (when all participants were smoking), cytisine reduced the perceived satisfaction and reward from smoking, as indicated by the scales of the mCEQ. Interestingly, the 2 participants who continued to smoke throughout the study also reported that smoking was less satisfying and less rewarding at the end of treatment compared with baseline. Therefore, smoking satisfaction and psychological reward were not found to be good predictors of abstinence in this study, but the study may indicate that cytisine can have a positive effect on these measures.

This study found that large between-subject variability exists in pharmacokinetics of cytisine. This study has also shown that that there is also variability in smoking cessation. Therefore, it was reasonable to ask whether variability in pharmacokinetics could, at least in part, explain the variability in smoking cessation. In other words, is there a relationship between plasma cytisine

concentration and effect? If so, would it be possible to predict who will be successful at quitting smoking just by looking at the cytisine concentrations? On day 1, it appeared that reduction in craving scores was highly correlated to the increase in plasma cytisine concentration. However, as the study progressed, craving for cigarettes continued to decrease despite the overall decline in plasma cytisine concentration thus there was no simple relationship between cytisine plasma concentration and cigarette craving. In addition, cytisine concentrations measured in participants did not appear to be related to quitting. Because this study failed to identify a relationship between plasma cytisine concentration and cigarette craving or smoking cessation, it was not possible to predict, based on plasma concentrations of cytisine alone, whether a participant would quit smoking during the study. Thus, the results showed that concentration-effect relationship may exist for cigarette craving on day 1 of cytisine treatment but there was no evidence to suggest there is such a relationship for the whole duration of the therapy.

It is important to discuss the setting of this study and how it may have affected results. In this study, participants were exposed to 2 distinct environments: day 1 and the rest of the study. On day 1, participants were in a very controlled environment (clinic setting) and therefore results in subjective measurements may not be a good reflection of a real world setting because it is likely that they were not exposed to the various cues that could potentially increase craving in the real world setting. It is also likely that on day 1 participants had more motivation to quit smoking and being around other people who also wanted to quit smoking may have influenced their subjective responses. In addition, although no deliberate counselling was offered to these participants, it is possible that being involved in the study and attending clinic visits where they had one-to-one, face-to-face contact with the investigator may have influenced their responses to subjective assessments. For the remainder of the study, participants were exposed to normal daily life situations and thus each participant may have been exposed to different environments which could have influenced their responses in the questionnaires which could not be fully assessed.

In general, self-reported smoking appeared to be reliable because the results from biochemical validation showed that mean cotinine levels decreased as the study progressed corresponding to the decreased number of participants smoking (self-report). However, biochemical validation also revealed that self-reporting was not always reliable because it relies on the honest reporting of individuals. Despite the limited number of participants (and close monitoring), one participant had not been honest about their smoking. Although, strictly speaking, this was not a smoking cessation study, this result may imply that not at all participants may have been honest in their other subjective experiences such as craving.

The most common side effects reported in the study included gastrointestinal disturbances, changes in cigarette taste and dreams/nightmare. Symptoms reported in this study were similar to those reported in the literature and many were symptoms associated with nicotine withdrawal (Tutka and Zatonski 2006, Hajek, McRobbie et al. 2013). Participants were not asked specific questions about adverse effects and were asked to report freely on anything that they found unusual while taking cytisine to encourage reporting of everything they had experienced (and not just the common symptoms). However, because a number of participants were taking part in the study at the same time, participants had the opportunity to talk amongst themselves and share what they had experienced. This may have resulted in increased reporting of the more common adverse effects than any uncommon ones.

A complex dosing regimen of cytisine was expected to be a barrier to adherence. In the study it was found that only one of the eleven participants recorded adhering to the dosing regimen although there is no way to verify this. Although tablets counts were done at every visit, this provided very limited information because it did not provide any information on how the tablets may have been taken or whether the participant strictly followed the dosing regimen. If participants with close monitoring found it hard to comply with the dosing regimen, it may be expected that in a less controlled setting, the adherence to cytisine treatment may be far worse.

Participants who take part in these studies may not be a true representation of the wider population of smokers, because they may be more motivated to quit smoking than other smokers in the population. In addition, the participants in this study may not be a good representation of the whole general smoking population because only individuals who had good general health (i.e. with adequate renal function) were included. Since it is clear from the current studies that cytisine is renally eliminated (Chapter 5), an important population to study in the future would be individuals with impaired renal function. It would be important to investigate whether there is increased cytisine concentration in these people and whether this leads to a greater incidence or severity of adverse effects.

Large variability may exist between individuals in self-report measures due to differences in rating style (Shiffman, West et al. 2004). In particular, withdrawal symptoms can be similar to general emotional distress. Ideally, a larger number of participants (such as large-scale population trials) would reduce bias for these measures.

One way to improve subjective assessments in these types of studies may be to use multiple questionnaires that combined the best elements of the currently available scales because there is no

"ideal" questionnaire that assesses different withdrawal symptoms and it has been shown that some scales measure certain withdrawal symptoms better than others (West, Ussher et al. 2006).

Another improvement in the study may involve including a questionnaire to specifically address questions about taking cytisine (e.g. how were your cigarette cravings after you swallowed your first tablet?). Also, a qualitative study to explore in depth of participants' experiences through one-to-one interviews may be useful in exploring what may have influenced their subjective measures.

5.12 Summary

In summary, cytisine appeared to be well tolerated in participants under normal dosing conditions. Cytisine may be effective in reducing cravings for cigarettes during the recommended dosing period and also may help stabilise withdrawal symptoms in individuals who have stopped smoking. On day 1 of the study where participants were kept in a relatively controlled environment, increases in cytisine plasma concentrations appeared to be highly correlated to the reduction in cravings for cigarettes. However, pharmacokinetic modelling showed that cytisine does not reach a steady state concentration following recommended dosing and reduction in the dosing frequency as the treatment progresses results in the overall decrease in cytisine plasma concentration. However, despite this decline in plasma concentration, mean cigarette craving continually decreased in the study. Thus there does not appear to be a simple relationship between cytisine plasma concentration and cigarette cravings.

Chapter 6 General discussion

6.1 Overview

The following discussion will review the major findings of the current research and discuss what these findings add to the existing literature, the limitations of the study and suggest future directions for research. The conclusions of the thesis are presented at the end of the chapter.

6.1.1 Aims of the thesis

The main aims of this thesis were:

1. To obtain basic pharmacokinetic data of cytisine in humans
2. To study the effects of cytisine on the physiological (blood pressure, pulse, breathing rate) and psychological measures (cigarette cravings, withdrawal, smoking satisfaction and mood) in human smokers
3. To explore whether some of these effects (in particular cigarette craving) measured in smokers could be related to the plasma concentrations of cytisine under normal dosing conditions.

6.2 Summary of main findings and discussion arising from previous chapters

Prior to this work, there were no published data on cytisine pharmacokinetics in humans. No methods had studied the commercial forms of cytisine (Tabex® or nicotine-free oral strips) and no method had quantified cytisine in clinical samples following administration of these products. In order to undertake pharmacokinetic studies in humans, sensitive analytical methods were developed and validated for the detection and quantification of cytisine in human plasma and urine (Chapter 3). These methods allowed the analysis of samples obtained from human smokers in subsequent studies.

Having established a validated assay, it was possible to examine the pharmacokinetics of cytisine in plasma following a single 3 mg dose in a small number of participants (n=7) (Chapter 4). Results from the pharmacokinetic analysis demonstrated that cytisine has a relatively simple pharmacokinetic profile. Cytisine reached its maximum concentration at 1-2 hours after an oral dose and the plasma elimination half-life of cytisine was approximately 4.8 hours (Jeong, Newcombe et al. 2014). This is much shorter than the half-life of varenicline (synthetic analogue derived from cytisine) (17-24 hours) (Burstein, Fullerton et al. 2006, Faessel, Smith et al. 2006).

Consistent with animal studies that have shown that cytisine administered is renally excreted (Tabex 2006), cytisine was detected as an unchanged drug in urine in humans (Jeong, Newcombe et al. 2014). *In-vitro* metabolism study with human liver preparations failed to detect any glucuronide or oxidised metabolites (Chapter 3). Consistent with this, the analysis of plasma and urine samples following a single dose also failed to detect any cytisine metabolites (Chapter 4). Similarly, much of varenicline administered is also excreted as an unchanged drug (more than 90% of administered dose) (Obach, Reed-Hagen et al. 2006). However, minor metabolites (N-carbomylglucuronide, hydroxy-varenicline, N-glucosylvarenicline) have been identified with varenicline in humans and it is currently unclear whether these are active or inactive (Obach, Reed-Hagen et al. 2006). If cytisine is not an enzyme substrate, pharmacokinetic interactions at the level of enzyme competition are unlikely to occur with many other prescription drugs or other smoking cessation drugs. This may have important implications for cytisine's use with other smoking cessation drugs in combinational therapy, which may be more advantageous than monotherapy (Ebbert, Hays et al. 2010). As for any smoking cessation medication, drug interactions can exist due to smoking and smoking cessation impacting on enzyme regulation and hence metabolic clearance of other drugs (Bertilsson, Carrillo et al. 1994). The most well-known example is CYP1A2 induction in smokers (Schrenk, Brockmeier et al. 1998). The prevalence of smoking is especially high in patients with alcohol and drug and mental health problems and therefore it is important to evaluate the risks of potential interactions between psychiatric medications and enzymes in these subpopulations of smokers (Lawrence, Mitrou et al. 2009).

People with schizophrenia have a very high rate of smoking (Bertilsson, Carrillo et al. 1994, Shirley, Hon et al. 2003). It has been shown that even smoking 7 to 12 cigarettes a day can lead to maximum induction of clozapine and olanzapine (2 drugs metabolised by CYP1A2 and extensively used in schizophrenics) metabolism (Haslemo, Eikeseth et al. 2006) and the mean plasma concentrations of these drugs are significantly lower in smokers than non-smokers (Rostami-Hodjegan, Amin et al. 2004). Upon smoking cessation, the change in mean half-life of CYP1A2 activity was reported to be

38.6 hours (Faber and Fuhr 2004). Smokers with schizophrenia frequently need larger doses of antipsychotic medications for a therapeutic effect and therefore smoking cessation in patients stabilised on these medications may lead to increases in drug concentrations and thus adverse effects.

Because cytisine undergoes minimal or no hepatic metabolism, cytisine pharmacokinetics should be unaffected in patients with hepatic insufficiency and thus could be a more useful drug for such individuals than smoking cessation drugs such as bupropion or nortriptyline which undergo significant hepatic metabolism (Venkatakrishnan, von Moltke et al. 1999, Hesse, Venkatakrishnan et al. 2000). In contrast, since cytisine relies on renal elimination, patients with severe renal impairment may have increased cytisine exposure and thus may require dose adjustment.

The results from pharmacokinetic analysis also showed that cytisine could be fit to a one-compartment model, suggesting that cytisine either remains in the blood compartment or distributes to other compartments at a very rapid rate. The volume of distribution of 115 L indicates that cytisine is not confined to the blood compartment. These data do not provide any evidence that cytisine can cross the blood-brain barrier in humans because the volume of distribution does not provide information regarding the tissues or organs to which the drug is distributed. Cytisine is thought to share a similar mechanism of action to varenicline in smoking cessation; both drugs are partial agonists of the neuronal $\alpha_4\beta_2$-nicotinic acetylcholine receptor (Rollema, Coe et al. 2007). In contrast to cytisine, varenicline concentrations in plasma decrease in a biphasic manner (i.e. fits a two-compartment model) (Ravva, Gastonguay et al. 2009) and animal studies show that there is good distribution of varenicline in the brain (Rollema, Chambers et al. 2007, Rollema, Shrikhande et al. 2010). In contrast, in rats, the brain penetration of cytisine has been shown to be poor (less than 30% of the plasma concentration) (Reavill, Walther et al. 1990). The results from the pharmacokinetic analysis show that there is no distinct distribution phase for cytisine, and this may suggest that either plasma concentrations are representative of other tissues including the brain (presumed site of action) or that cytisine may not cross the blood-brain barrier. Another alternative explanation is that although there is some distribution of cytisine into the brain, the extent of this distribution is so small such that the cytisine plasma pharmacokinetics appears to be unaffected. This may, however, simply reflect species differences in the ability of cytisine to cross the blood brain barrier in humans compared with rats. A more accurate method to animal studies would be to use Positron Emission Tomography (PET) scanning in humans to determine the distribution of cytisine (radio-labelled) in the body. This would allow the investigation of cytisine's distribution in real time and allow the determination of which tissues (e.g. brain) are exposed to cytisine.

Unfortunately, this method is costly and the equipment to carry out these experiments is not widely available.

The results from the single-dose study (Chapter 4) demonstrated that 3 mg administration of cytisine was well-tolerated in healthy smokers despite being double the normal amount that is recommended to be ingested at one time. An interesting feature of this study was the small between variability observed in the plasma cytisine concentrations. This may have been due to the small variability in participant demographics (all participants were all males, relatively fit and young smokers, renal clearance etc., a full list of exclusion/inclusion criteria are presented in Chapter 4).

Following the single-dose pharmacokinetic study of cytisine, the next logical step was to investigate the multi-dose pharmacokinetics of cytisine in human smokers to determine whether there is drug accumulation, whether there are changes to the pharmacokinetics of cytisine and whether there are any changes to the metabolism of cytisine in humans. The subsequent multi-dose study (Chapter 5) therefore involved participants who were instructed to adhere to the manufacturer's dosing regimen guidelines. Results from the multi-dose study showed that on day 1, plasma concentrations increased after 4 doses then appeared to plateau in participants. However, cytisine concentrations continually declined after the last dose of the first day (6^{th} dose) until the next dose was taken on day 2. This showed that there was very little accumulation of cytisine from day to day with the current dosing regimen. This is a major difference from varenicline therapy, where there is large between day-to-day accumulation and a steady state concentration is reached within 4 days of treatment that is maintained during the 12 week course of treatment (Ravva, Gastonguay et al. 2009). There are no head-to-head trials between cytisine and varenicline so it is unknown which treatment is better at achieving long-term abstinence rates among smokers. Whether maintaining a steady state concentration (for the whole duration of therapy) is associated with a greater reduction in craving or whether it increases the chance of achieving long-term abstinence in smokers is unknown. Given that cytisine did not reach a steady state concentration that is maintained for the whole duration of the therapy, it would be worthwhile investigating whether maintaining a steady state concentration with cytisine improves the long-term abstinence rates.

Pharmacokinetic data from the single dose study were combined with the data from the multi-dose study to model the plasma drug concentration throughout the 25 day period. In contrast to the single dose study, the measured plasma cytisine concentrations on day 1 and the modelling data showed far greater between subject variability, with more than 2-fold differences in maximal measured concentrations. Over the time course of the study, plasma concentrations followed this

trend, and population pharmacokinetic modelling consistently under- or over-predicted an individual's plasma cytisine concentration. Because there was more between subject variability in terms of age range, sex (both males and females) in the multi-dose study compared with the single-dose study, the results from the multi-dose study are likely more representative of the general target population of smokers than the single dose study. Cytisine plasma concentration and mean craving scores on day 1 appeared to be highly correlated.

For the remainder of the study, pharmacokinetic modelling showed that cytisine did not reach a steady state concentration and there was a decrease in peak plasma cytisine concentrations due to dose tapering. Despite this decrease in plasma cytisine concentration, the mean craving scores continually declined and there did not appear to be a simple relationship between cytisine plasma concentration and cigarette cravings. It appears that there is a time-dependent change in cravings (cravings decrease as time on cytisine therapy increases) which suggests that the craving a person experiences is a product of more than just pharmacological factors – e.g. environmental cues and personality variables (Cinciripini, Wetter et al. 2003, Cosci, Corlando et al. 2009).

Pharmacokinetic parameters estimated by combining the data from the single dose study and the multi-dose study produced results which were very similar to the results of the single dose study alone indicating that there were no dose-dependent or time-dependent changes to the pharmacokinetics of cytisine.

The results of the multi-dose study also demonstrated that, excluding craving for cigarettes, withdrawal symptoms (measured by the Mood and Physical Symptoms Scale) remained stable during the course of the treatment, even in participants who had stopped smoking. No significant changes to mood (total mood disturbance) were observed. This may be important since smoking cessation has been associated with depression (Kahler, Brown et al. 2002).

Although the multi-dose study was not designed to be a smoking cessation study, for instance with respect to statistical power, it was possible to determine smoking cessation. In addition to the variability observed in pharmacokinetics, this study, as with other such studies, showed that there is also variability in smoking cessation (not all participants quit smoking), but there did not appear to be a simple relationship between pharmacokinetics in these participants and cessation.

The current standard cytisine dosing regimen includes a target quit day (day 5), but in line with actual practice, participants were not prevented from smoking, thus had nicotine exposure. Craving has been shown to be relieved to some extent using nicotine replacement therapy which suggests

that it is partly driven by nicotine depletion (Shiffman, Ferguson et al. 2006). Therefore nicotine exposure may have confounded any simple relationship between plasma cytisine concentration and cigarette craving. In order to examine the relationship between cytisine concentration and its effects on cigarette craving and withdrawal, it would be worthwhile to investigate simultaneously the nicotine and cytisine concentrations; this may provide a better understanding of the complex relationship between drug concentration and craving. It would require the development of an analytical assay that can determine both cytisine and nicotine, and would be useful for future studies. However, at the same time, craving also has a circadian component that is not influenced by nicotine depletion (e.g. NRT has been shown to have little control over diurnal fluctuations in craving (Teneggi, Tiffany et al. 2002)) which suggests that not all components of craving are directly controlled by, or related to, nicotine levels. Circadian variations in craving may be triggered by environmental cues and other numerous factors including negative affect such as psychological stress (Erblich, Boyarsky et al. 2003, Childs and De Wit 2010), emotional regulation (Szasz, Szentagotai et al. 2012), expectancy to smoke (McBride, Barrett et al. 2006) and other factors such as expectancy in drug (participants expecting that the drug will work) (McBride, Barrett et al. 2006), therapeutic relationships (influence of the investigator on the participants in the study) (Sapolsky 1965) and alcohol intoxication (Burton and Tiffany 1997, Sayette, Martin et al. 2005). Craving can also significantly reduce when participants focus on the long-term negative consequences of smoking (Kober, Kross et al. 2010). Individuals may have used different strategies to cope with craving, and such strategies have not been explored in the study. There is evidence that cigarette availability can also influence craving to smoke (Carter and Tiffany 2001). Participants in this study may have had continued access to cigarettes and knew they could smoke if they wanted to, which again could have affected their subjective responses. Thus, in addition to measuring nicotine levels in participants, features to include in future studies might be questions on expectancies regarding quitting, cigarette availability, and potential environmental craving triggers during the study period. However, given that craving is a complex phenomenon with many components, involving pharmacology and psychological factors that may be driven by environmental cues (Colamussi, Bovbjerg et al. 2007) and placebo effect, a clear relationship between craving and cytisine concentration may still be difficult to assess. To complicate things further, the nature and time course of these responses and symptoms can be widely variable among individuals (Hatsukami, Hughes et al. 1984).

Because craving may be influenced by multiple factors, this leads to the question of how studies measuring craving for cigarettes could be conducted. In order to minimise environmental factors,

smokers may have to be kept in a lab-setting throughout the whole course of cytisine treatment to minimise otherwise uncontrollable situations. Of course, this would not be a true representation of the craving response in the real world setting. If craving is to be a measure in a real-world setting, real-time data collection (e.g. mobile phones) may provide more frequent assessments of mood and craving. However, it would not be feasible to take a blood sample as frequently along with these measures.

Since individuals who take cytisine may not completely abstain from smoking, it would be important to examine what influence nicotine could have on the pharmacokinetics of cytisine. It would be predicted that there would be no interaction due to competition for enzymes because cytisine does not undergo metabolism. Results from pharmacokinetic modelling suggest that nicotine is unlikely to have an effect on cytisine pharmacokinetics because no distinct changes in the pharmacokinetics were observed when participants quit smoking or reduced the number of cigarettes smoked.

The findings from the multi-dose study add to the literature on the potential adverse effects of cytisine. The most common self-reported side effects in the current multi-dose study were changes in appetite, dry mouth, cigarettes tasting less pleasant, dreams/nightmares and irritability. These side effects are similar to those that have been reported in the literature (Tutka and Zatonski 2006, West, Zatonski et al. 2011). Cytisine appears to be generally well-tolerated in participants who follow the recommended dosing regimen. However, most of these self-reported side effects including irritability, appetite changes and sleep disturbances can also result from nicotine deprivation (Maliszewski and Straczynski 1972, Zatonski, Cedzynska et al. 2006). Overall, cytisine appeared to be generally well-tolerated.

Finding from the multi-dose study, in which only 1 participant strictly adhered to the dosing regimen, add to previous findings that there is poor medication adherence reported with the current recommended dosing regimen (Walker, Howe et al. 2014). Other earlier trials of cytisine have also reported less than 100% adherence (Scharfenberg, Benndorf et al. 1971, Walker, Howe et al. 2014). It has been reported that even with simple once-daily dosing, not all participants in studies are fully adherent to the dosing regimen (Claxton, Cramer et al. 2001). This suggests that in the real-world setting, the complex dosing regimen of Tabex® may be a disadvantage over other smoking pharmacotherapies that have a simpler dosing regimen (e.g. varenicline). Therefore, it may be worthwhile exploring new dosing regimens that involve fewer numbers of cytisine tablets per day.

6.3 Limitations

A number of possible limitations in the study design have been discussed in the previous chapters. Because this work is a preliminary study into the pharmacokinetics of cytisine in human smokers, a limitation is the modest number of participants. This is a particularly important issue in exploring the relationships between plasma cytisine concentration and the effects in psychological measures, such as cigarette cravings. The questionnaires used in the study to assess psychological measures are validated tools commonly used throughout the literature (Cox, Tiffany et al. 2001, West and Hajek 2004, West and Ussher 2010). These tools are also used clinically to explore changes in the severity of withdrawal symptoms and craving in individuals over a time period. However, because they are used to report subjective outcomes, large variability may exist between individuals and thus studies should involve a relatively larger sample size in order to look for general trends and patterns across a population of smokers.

This problem highlights a major difficulty in undertaking such research; pharmacokinetics is an objective measure and as shown in the single does study, even with a small number of people (7 people), it was possible to generate very precise data. With the data obtained from the single dose and multi-dose study, it was possible to generate a population pharmacokinetic model. However, to study the psychometric effects of drugs, such as craving, a relatively large sample size is required as discussed above. Designing and conducting studies that embrace pharmacokinetics and psychometric effects of smoking therefore remains a challenge.

For many pharmacokinetic investigations, it is not always possible to investigate the pharmacokinetics of drugs at the site of action. Although plasma concentrations of cytisine provide useful information such as determining how long cytisine remains in the body, the concentration of cytisine in the brain (presumed site of action) may be more useful in determining the concentration-effect relationships of cytisine. Thus it would be important to understand the distribution of cytisine to the brain.

The widely accepted main outcome for smoking cessation medications is continuous smoking abstinence (e.g. less than 5 cigarettes smoked at >6 months (West, Hajek et al. 2005)). However, it is difficult to conduct rigorous pharmacokinetic-pharmacodynamic studies using long term smoking abstinence because it is not directly measurable at the time of pharmacokinetic studies. Therefore long term continuous abstinence is an impractical pharmacodynamics measure for short-term (pharmacokinetic) studies. Surrogate outcomes to predict long term smoking abstinence, such as reduction in cravings were therefore used in the thesis. Craving is an important nicotine withdrawal

symptom (Hughes and Hatsukami 1986, Killen and Fortmann 1997, Shiffman, West et al. 2004, Hughes 2007) but there are limitations to using craving as a measure to study concentration-effect relationships because it a complicated measure that may be driven by many factors other than pharmacology (as discussed in the previous section). Further feedback from participants in the trial and qualitative sub-study to determine the influence of participant in taking part in such trials would be worthwhile to study in the future.

Because the studies in this thesis involved a small number of participants, it is very unlikely that it would be able to pick up rare side effects.

As these studies had strict inclusion and exclusion criteria, the participants are not a true representation of the whole smoking population; for example, patients with impaired renal function and smokers with co-morbidity disorders were excluded.

6.4 Future directions

At this time, it is not clear that there is either a simple or clinically meaningful relationship between plasma concentration of cytisine and cigarette craving, withdrawal or mood in human smokers who want to quit smoking.

Preliminary studies of the pharmacokinetics of cytisine in smokers have shown that there is large between-subject variability. From the findings from this thesis, the following questions have arisen that warrant further research:

1. What biological factors (covariates, e.g. body mass index) contribute to between-subject variability in the pharmacokinetics of cytisine?

Understanding biological factors that may contribute to between-subject variability in pharmacokinetics of cytisine may be useful in determining reasons for higher than expected concentrations of cytisine and thus improve the safety of the drug in individuals. Conversely, understanding factors that may contribute to lower than expected concentrations could be useful in improving the therapeutic outcome of cytisine in individuals. In order to address between subject variability, it would be necessary to recruit a large number of participants.

One important factor to study would be renal function. As this study was a preliminary study, it only included relatively healthy participants and explicitly excluded individuals with impaired renal function. As renal elimination appears to be an important excretion mechanism for cytisine, impaired renal function may affect the drug's pharmacokinetics. Studying the extent that renal impairment has on the pharmacokinetics will help to determine whether the dosing regimen needs to be adjusted in such patients. Other biological factors such as weight and height, age and sex will also need to be explored to refine pharmacometric modelling.

2. What is the influence of dose, dosing frequency and dosing duration on the pharmacokinetics of cytisine?

As the findings from this thesis suggest, there does not appear to be a pharmacokinetic rationale for the current recommended dosing regimen of Tabex®. The pharmacokinetic data obtained from the work in this thesis can be used to develop an improved dosing regimen. The complex dosing regimen of Tabex® appears to result in poor adherence to treatment, as shown in the multi-dose study, despite relatively intense monitoring of participants (tablet counts at each visit, dosing diary, reminders whenever there is a change to the dosing regimen). An improved dosing regimen may enhance the effectiveness of cytisine for smoking cessation by improving adherence. First of all, a single dose escalation study could be conducted to determine the influence of dose on plasma concentration and tolerability of cytisine in smokers. Since the single dose study in this thesis explored a dose of 3 mg, it would be worthwhile investigating the pharmacokinetics of cytisine following an administration of a single Tabex® tablet (1.5 mg) and also a higher dose (e.g. 4.5 mg; 3 tablets of Tabex®) to determine whether dose has an influence on pharmacokinetic factors such as the maximum concentration and drug exposure ($AUC_{0\text{-}12h}$). It would also allow the determination of concentration-related adverse effects of cytisine. Further to this study, it would also be of interest to investigate the effects of dosing interval on the plasma concentration of cytisine and whether a change in dosing interval could lead to a steady state concentration being reached and maintained for the duration of cytisine treatment. For example, instead of the standard dosing regimen where a single Tabex® tablet (1.5 mg cytisine) is given every two hours six times a day (during the first 3 days of the treatment), two tablets (3 mg) might be given every four hours for three times a day or three tablets (4.5 mg) might be given every 6 hours twice a day to see whether this influences the time to reach steady state concentration in plasma. If maintaining a steady state concentration is possible with cytisine it would be also worthwhile conducting studies that could extend the therapy duration

for cytisine (e.g. 12 weeks like varenicline) such that head-to-head comparisons with varenicline for smoking cessation are more feasible. Before this however, the effect of dosing duration on the plasma concentration of cytisine would need to be studied, as well as the tolerability of cytisine when dosing is adjusted and maintained at a steady state plasma concentration for a longer period of time.

3. What are the transporters involved in renal elimination of cytisine?

Identifying renal transporters of cytisine will help the understanding of the biological processes that contribute to cytisine's distribution and elimination. As cytisine has been shown to be renally eliminated as an unchanged drug, it may be a substrate for transporters in the kidney, similar to varenicline, which has been shown to be a substrate for organic cationic transporter (OCT2) (Obach, Reed-Hagen et al. 2006). In addition, a large volume of distribution of cytisine (115 L) suggests that cytisine distributes to other compartments (currently unknown) which may also be influenced by drug transporters. Studies might explore whether cytisine could inhibit the transport of other co-administered drugs or whether co-administration of other drugs could influence the renal elimination of cytisine (potential for drug interactions) and to which tissues may or may not be exposed to cytisine. Understanding cytisine disposition may help gain a better understanding of cytisine's mechanism of action for smoking cessation.

4. What is the influence of delivery method/formulation?

With the growing popularity of alternative methods of nicotine delivery, such as oral strips and vapourisers, exploration of formulations other than oral tablets would also be of interest, in terms of outcome for smokers wanting to quit smoking. A worthwhile exercise would be to develop and test electronic-cigarettes (e-cigarette) which deliver cytisine. This would allow much of the cytisine to be delivered straight to the central nervous system with minimised systemic effects. Furthermore, because the oral tablet does not replicate any of the behavioural aspects of smoking, a cytisine e-cigarette may be expected to obtain better outcomes for smoking cessation and reductions in craving. It would be interesting to investigate whether a clear concentration-effect relationship in smokers with cravings can be shown in smokers.

6.5 Conclusions

This thesis includes work that allowed the publication of novel human cytisine pharmacokinetic data (Jeong, Newcombe et al. 2014). Preliminary studies have been conducted in smokers to obtain basic pharmacokinetic data and cytisine's effect on withdrawal, cigarette craving, smoking satisfaction and mood were measured to examine whether these could be related to the plasma concentrations in smokers following the current standard dosing regimen of Tabex®. This work has shown that cytisine is pharmacokinetically different from varenicline, its synthetic analogue. The major findings of this work are:

1. Cytisine reaches a peak plasma concentration around 2 hours following a single dose and has an elimination half-life of approximately 4.8 hours
2. Cytisine is eliminated in urine as an unchanged drug and no metabolites have been detected
3. Following the recommended dosing regimen, cytisine does not reach a steady state at any point during therapy
4. Population pharmacokinetic modelling shows that there is large between-subject variability in cytisine pharmacokinetics
5. Cytisine appears to be effective in reducing craving for cigarettes and may stabilise withdrawal symptoms even in individuals who stop smoking
6. Cytisine appears to be generally well-tolerated under normal dosing and no serious adverse effects have been identified
7. There does not appear to be a simple relationship between cytisine plasma concentration and effects on cigarette craving.

Rigorous research is still required to determine the therapeutic and toxic plasma concentrations for cytisine in smokers. The information gained from conducting these studies will be used to inform the design of future trials of cytisine that explore different dosing regimens. More research is warranted to explore ways to improve the clinical effectiveness of cytisine for smoking cessation.

Appendices

Appendix 1:	Ethics and SCOTT approval
Appendix 2:	Study Consent form
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Appendix 1 Ethics and SCOTT approval

Health and Disability Ethics Committees

Northern X Regional Ethics Committee
Private Bag 92522
Wellesley Street
Auckland 1141
Phone: (09) 580 9105
Fax (09) 580 9001
Email: northernx_ethicscommittee@moh.govt.nz

22 December 2011

A/Professor Janie Sheridan
School of Pharmacy
University of Auckland
Private Bag 92 019
Auckland 1142

Dear Janie

Re: Ethics ref: **NTX/11/05/038** (please quote in all correspondence)
Study title: Pharmacokinetic and dose response characteristic of cytisine – C-DRAKS studies. Protocol, v#1, 8/8/11; Prot/Amend v#2, 12/12/11; PIS/Cons v#4, 12/12/11
Investigators: Associate Professor Janie Sheridan (Principal), Dr David Newcombe, Dr Malcolm Tingle, Dr Natalie Walker, Soo Hee Jeong

Thank you for your letter dated 20 December 2011 with amendments to the study.

The changes and documentation were reviewed by the Deputy Chairperson of the Northern X Regional Ethics Committee under delegated authority.

Ethical approval is granted to:

- Change to inclusion criteria
- Protocol amendment for C-DRAKS Single dose study [version 2, dated 12.12.2011]
- Protocol amendment for C-DRAKS Multi dose study [version 2, dated 12.12.2011] – please **replace V-3 with V-2** (on footer) as indicated in cover letter and as for single dose study.
- Information sheet/Consent form for C-DRAKS 1 version [4, dated 12.12.2011] – please amend consent form from **V3 to V4** as for Information sheet as they are treated as one document.
- Information sheet/Consent form for C-DRAKS 2 version [4, dated 12.12.2011] – please amend consent form from **V3 to V4** as for Information sheet as they are treated as one document
- Advertisements with mobile numbers inserted (received 20/12/11)

Yours sincerely

Cheh Chua
Administrator
Northern X Regional Ethics Committee

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A BUSINESS UNIT OF
THE MINISTRY OF HEALTH
www.medsafe.govt.nz

24 August, 2011

TT50-8671
(1219)

Assoc. Prof. Janie Sheridan
University of Auckland
Private Bag 92019
AUCKLAND 1

Dear Associate Professor Sheridan

Clinical Trial on: Tabex (Cytisine)
Protocol Number: Version 1

Further to your email letter of 22 August 2011, SCOTT would like to thank you for your revised application for the single dose and multiple dose studies.

This trial is now recommended for approval subject to you being willing to exclude subjects with renal impairment (estimated GFR < 30 ml/min). The reason being that cytisine is cleared by renal route and it is reasonable to assume that severe renal impairment will result in excessive concentrations.

SCOTT also recommends that the applicants measure standard Haematology, Biochemical and dipstick urine before enrolling volunteers and at the end of the study. Apart from this data being able to exclude subjects with renal impairment it will protect the applicant from enrolling medically unsuitable volunteers.

Could you please let Becci Slyfield at the address below know whether you are willing to exclude these subjects so that a final approval letter can be arranged.

Yours sincerely

Dr Alexander Bolotovski
for Director-General of Health

Level 6 Deloitte House 10 Brandon Street PO Box 5013 Wellington Phone (04) 496 2000 Fax (04) 819 6806

Appendix 2 Study consent form

Cytisine as a smoking cessation aid: measuring blood levels, craving, withdrawal and mood over a course of treatment CDRAKS2

CONSENT FORM

- I understand the information sheet (updated 21.09.2012) for volunteers taking part in this study, which has been read and discussed with study researchers. The study is designed to look at how the medicine works in my body and whether it can help me quit smoking.
- I have had the opportunity to discuss this study with study researchers and I am satisfied with the answers I have been given.
- I have had the opportunity to use whānau/family support or a friend to help me ask questions and understand the study.
- I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my future health care/continuing health care from smoking cessation programmes or other health providers.
- I understand that my participation in this study is confidential and that no material that could identify me will be used in any reports on this study.
- I understand the compensation provisions for this study.
- I have had time to consider whether to take part.
- I know whom to contact if I have any questions about the medication used in this study or about the study in general.
- I understand that there will be no personal details that identify me (such as my name, address) on the questionnaires.
- I understand that any data collected as part of this study will be stored securely for 15 years at the School of Pharmacy, The University of Auckland, in accordance with the Privacy Act, 1994. After this time the information will be safely destroyed.
- I understand that any information collected, as part of this study will not be used for any other purpose, without my permission and ethical approval, nor given to any other third party outside of the research team.
- I understand that there may be a significant delay between data collection and publication of the results.
- I wish to receive a copy of the results
  YES/NO

- I wish to receive a copy of my data

  YES/ NO
- I want my excess/unused biological samples (urine & bloods) returned to me

  YES/ NO

I _____ (print full name)

of _____ (print address)

_____

_____

hereby consent to take part in this study.

Verbal consent of participant received? Yes / No (circle response)

Date: ___/___/___
day/month/year

Full names of researcher: _____

Contact phone number: _____

Project explained by: _____

Signature: _____

Date: ___/___/___
day/month/year

Ethical Approval

This study has received ethical approval from the National Multi-region Ethics Committee, ethics reference number NTX 11.0.5.038

Appendix 3 Participant Information Sheet (PIS)

Single dose study PIS

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND

School of Pharmacy
93 Grafton Road
Grafton
Private Bag 92019
Auckland
NEW ZEALAND
Telephone: 64 9 373 7599
Facsimile: 64 9 367 7192
Email: j.sheridan@auckland.ac.nz

Cytisine as a smoking cessation aid:
single dose study
(C-DRAKS-1 study)

PARTICIPANT INFORMATION SHEET

You are invited to take part in a study which is exploring how a new smoking cessation medicine works in the body. By taking part you will be involved in a study that will help a university student obtain a higher degree (i.e. their PhD). To help you make a decision about participating in the study, we ask that you read this information sheet.

Who is co-ordinating this study?
The study is run by the School of Pharmacy at the University of Auckland.

What is the aim of this study?
The aim of this study is to see how much of the drug is in the blood and urine after a single dose and what impact it has on craving for cigarettes and mood and to explore any side effects.

Why have I been selected?
You have been selected to take part in this study because you have contacted the research team, and said that you were willing to participate in research. You also meet the requirements below:

- You are currently a regular smoker.
- You are 18 years of age or older.
- You are able to provide written consent.

You cannot take part in this study if you have any of the following conditions:

- You are pregnant or breastfeeding
- You have had a stroke or heart attack or severe angina in the last two weeks
- You have diagnosed severe/uncontrolled hypertension (systolic blood pressure greater than 150mmHg, diastolic greater than100mgHg)
- You have severe renal impairment
- You have been diagnosed with schizophrenia
- You have been diagnosed with phaeochromocytoma
- You are a current user of NRT products (e.g. patches, gum, lozenges etc)
- You only smoke non-cigarette tobacco products (e.g. pipes, cigars)
- You are currently using non nicotine-based medicines such as Zyban (buproprion), clonidine, nortriptyline or Champix (varenicline)
- You enrolled in another smoking cessation programme (eg Text2Quit) or another cessation study (eg Fit2Quit).

Where will the study take place?
The study will take place at the School of Pharmacy, 93 Grafton Road in Grafton, Auckland or the School of Population Health, Morrin Road, Tamaki Campus.

How long will the study take?
The study will take three days.

How many people will be recruited into the study?
We are looking to recruit 7 smokers.

What is involved if I take part?
If you are eligible, a researcher will ask you to sign a written consent form.

If you agree to take part in the study, you will have your blood pressure and pulse taken, and a blood sample taken (10mls) on several occasions (see Table below). A saliva test strip (e.g. NicAlert® cotinine test strips) will be used to confirm your smoking status.

You will also be asked to complete a number of short questionnaires about how you are feeling, including your craving for cigarettes, any effects of withdrawal from smoking, and mood. You will then be given a single dose of the study drug – cytisine 3mg. You will also have blood samples taken at 15 minutes, half an hour, 1, 2, 3, 4, 6 and 8 hours. You will be asked to complete the questionnaires again at regular intervals. You will be asked to come back to the School of Pharmacy to have a blood and urine sample taken 24 hours after the first dose, and to complete the questionnaires again. You may also be asked to come back after 36 and 48 hours to repeat this. You will be provided with food and drink while at the School of Pharmacy.

What will I be asked at each visit?
At screening, a saliva test will be used to confirm smoking status, and a blood and urine sample will be taken to see how well your kidneys are working.

At each following visit, you will be asked to complete a set of other questionnaires that ask you about your craving for cigarettes, signs of withdrawal from smoking, your current mood and any side effects you may have experienced. At each visit, there will always be someone to help you if you have any queries or problems in completing the questionnaires. Blood and urine samples will be taken.

Summary of Visits to School of Pharmacy

Visit	What will happen	Questions asked	How long will you be at the School of Pharmacy?
Screening	We will ask you some questions to check if you can join the study. A blood sample and urine dipstick test will be taken. A saliva test will be used to confirm smoking status	Questions to determine • your level of nicotine dependence • how satisfying you currently find smoking	1 hour
1	If you are eligible you will be asked to sign a written consent form Bloods taken at 0 (just before dosing), 15 minutes, 30 minutes, 1, 2, 3, 4, 6, 8 hours after the first dose. Blood pressure, pulse and breathing rate measured at 0, 2, 4, 8 hours post dose. Spontaneous urine collection	Questions to determine • how much you have an urge to smoke (asked at 0, 1, 2, 3, 4, 6, an 8 hours) • mood (asked at 0, 2, 4, 8 hours)	9 hours
2	A single blood and urine sample will be collected from you, and your blood pressure, pulse and breathing rate will be measured	Questions to determine • how much you have an urge to smoke • mood	1 hour
3	As above	As above	1 hour
4	As above	As above	1 hour

Will there be any costs involved?
The study will require you to spend one day at University and to visit the University on the following day and possibly the day after. You will be provided with petrol vouchers to compensate you for any travel expenses and we are also offering a small reimbursement of vouchers worth $100. This will be paid at the end of your time in the study

What are the risks and benefits of this study?

Possible benefits
The study only involves taking a single dose of the study drug. However, you will have your blood pressure and pulse taken, and will have an opportunity to discuss quitting smoking if you wish.

Possible risks
Cytisine is a medicine derived from a plant; *Cytisus laburnum* (Golden Rain) which has been used in Europe for over 40 years as a smoking cessation product. It does not contain any nicotine, but is similar in structure to nicotine. It is currently not available for sale in New Zealand. If you take more than the recommended dose of cytisine you may experience nausea (feeling sick), vomiting, pupil dilation, a racing heart rate and general weakness. These feelings will disappear if you reduce the amount of cytisine you are taking to the recommended levels. Cytisine should not to be taken by women who are pregnant or breastfeeding. It is considered safe and does not affect your ability to drive or operate machinery.

The risks associated with taking blood from your arm are small, and may include bleeding, fainting, light-headedness, bruising, and/or an infection.

Your GP will be contacted if we find any abnormal results from your blood, urine or blood pressure tests, or in the unlikely event you have any negative reaction to the medicine.

Blood and urine samples

Blood and urine samples will be taken at screening to determine your kidney function. They will also be taken on study days to analyse what the body does to the drug once it is taken by you. All samples will be handled at the Auckland University.

If you wish to have any unused (excess) samples returned to you, you can indicate this on the consent form. Otherwise, blood and urine samples will be destroyed after analysis - the blood will be sterilized using steam, then buried. The urine samples will be flushed away).

Compensation
In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by the Accident Compensation Corporation (ACC) legislation. ACC cover is not automatic and each case is assessed by ACC, according to the provisions of the 2001 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are a wage earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, please contact your nearest ACC office or ask us for more information before you agree to take part.

Will the information about me be kept confidential?
The study files and all information that you provide will remain strictly confidential (private). No material that could personally identify you will be used in any reports on this study. The information will be kept securely at the School of Pharmacy, The University of Auckland and destroyed after 15 years according to national research guidelines. All computer records will be password protected. All future use of the information collected will be strictly controlled in accordance with the Privacy Act, 1994.

During the study, ethics committee representatives, study personnel, and members of the research team may review the information collected. This will only be done to check the accuracy of the information collected for the study and the information will remain confidential.

When will the results be available?
This study may take six months to run, so results may not be available until 2012. You will be asked if you would like to be sent a copy of the results of the study when you agree to participate.

Has the study received ethical approval?
Yes, this study has received ethical approval from the Northern X Regional Ethics Committee, which reviews Regional studies. Ethics reference number: NTX/11/05/038

What are my legal rights?

Your participation in this study is entirely voluntary (your choice). You do not have to take part. If you choose not to take part in this study you or your future healthcare will not be affected in any way. You may withdraw from the study at any time, without having to give a reason. Your withdrawal from the study will not affect your future health care from the smoking cessation programme or any other health service. You are encouraged to ask questions at any time during the study. If you have any questions please ask a study research assistant or contact:

Associate Professor Janie Sheridan,
Principle Investigator,
School of Pharmacy,
Faculty of Medical and Health Sciences,
The University of Auckland,
Private Bag 92019,
Auckland.
Ph: (09) 373 7599 extension 85247
Fax: (09) 367 7192
Email: j.sheridan@auckland.ac.nz

If you have any questions or concerns regarding your rights as a participant in this study you may wish to contact a Health and Disability Advocate at the Health Advocates Trust:

Northland to Franklin ph 0800 555 050

Study Investigators at the Faculty of Medical and Health Sciences, University of Auckland

- Associate Professor Janie Sheridan, School of Pharmacy
- Associate Professor Malcolm Tingle, Department of Pharmacology and Clinical Pharmacology
- Dr David Newcombe, School of Population Health
- Dr Natalie Walker, School of Population Health

Please keep this sheet for your information.
Thank you for taking the time to read about this study.

Multi-dose study PIS

Cytisine as a smoking cessation aid: measuring blood levels, craving, withdrawal and mood over a course of treatment (CDRAKS-2 study)

PARTICIPANT INFORMATION SHEET

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND

School of Pharmacy
93 Grafton Road
Grafton
Private Bag 92019
Auckland
NEW ZEALAND
Telephone: 64 9 373 7599 extn 85247
Facsimile: 64 9 367 7192
Email: j.sheridan@auckland.ac.nz

You are invited to take part in a study which is exploring how a new smoking cessation medicine works in the body and whether it has any effect on your craving for cigarettes. By taking part you will be involved in a study that will help a university student obtain a higher degree (i.e. their PhD). To help you make a decision about participating in the study, we ask that you read this information sheet.

Who is co-ordinating this study?
The study is run by the School of Pharmacy at the University of Auckland.

What is the aim of this study?
The aim of this study is to see how much of the drug is in the blood and urine during a course of treatment, and what impact the treatment has on craving for cigarettes, cigarette withdrawal and mood.

Why have I been selected?
You have been selected to take part in this study because you have contacted the research team, and said that you were willing to participate in research. You also meet the requirements below:

- You are currently a regular smoker.
- You have moderate/heavy nicotine dependence determined by the Fagerstrom Test for Nicotine Dependence.
- You are willing to try to quit smoking
- You are 18 years of age or older
- You are able to provide written consent.

You cannot take part in this study if you:

- Are pregnant or breastfeeding
- Have had a stroke or heart attack or severe angina in the last two weeks
- Have diagnosed severe/uncontrolled hypertension (systolic blood pressure greater than 150mmHg, diastolic greater than100mgHg)
- You have severe renal impairment
- Have been diagnosed with schizophrenia
- Have been diagnosed with phaeochromocytoma
- Are a current user of NRT products (e.g. patches, gum, lozenges etc)
- Only smoke non-cigarette tobacco products (e.g. pipes, cigars)
- Are currently using non nicotine-based medicines such as Zyban (buproprion), clonidine, nortriptyline or Champix (varenicline)
- Are enrolled in another smoking cessation programme (eg Text2Quit) or another cessation research study (eg Fit2Quit or CASCAID).

Where will the study take place?
The study will take place at the 93 Grafton Road in Grafton, Auckland or the School of Population Health, Morrin Road, Tamaki Campus.

How long will the study take?
The study will run for 26 days.

How many people will be recruited into the study?
We are looking to recruit 12 smokers.

What is involved if I take part?
If you are eligible, a researcher will ask you to sign a written consent form.

If you agree to take part in the study, you will be provided with some smoking cessation tablets called Tabex®, which contain cytisine. You will be asked to take the tablets in the following way:

- Days 1-3: 1 tablet every 2 hours through the waking day (up to six tablets per day)
- Days 4-12: 1 tablet every 2.5 hours (up to 5 per day); designated Quit date is day 5
- Days 13-16: 1 tablet every 3 hours (up to 4 per day)
- Days 17-20: 1 tablet every 4-5 hours (3 per day)
- Days 21-25: 1 tablet every 6 hours (2 per day)

You will be asked to quit smoking on day 5 of the treatment.

You will need to come to the University on day 1 to have your blood pressure, pulse and breathing rate measured and a small blood sample taken (6mls) on several occasions (see Table below). A saliva test strip (e.g. NicAlert® cotinine test strips) will be used to confirm your smoking status. You will be given your Tabex tablets and asked to start taking them. You will also have blood samples (6mls) taken at 2, 4, 6, 8, 10 hours after the first dose.

A urine sample will be collected from you throughout the day, when possible.

You will be asked to complete a number of short questionnaires about how you are feeling, including your craving for cigarettes, any effects of withdrawal from smoking, and mood. Your blood pressure, pulse and breathing rate will be measured at regular intervals.

You will be provided with food and drink during the day. In total you will need to remain at the School of Pharmacy for 10 hours.

You will be asked to come back to the School of Pharmacy for the day to have more blood and urine samples taken 24 hours after the first Tabex tablet, and to complete the questionnaires again. After that you will need to come to the School of Pharmacy on thirteen more occasions - on days 2, 3, 4, 5, 6, 13, 14, 17, 18, 21, 22 for one hour, on day 25 for six hours and on day 26 for one hour. .

What will I be asked at each visit?
On the screening day you will be asked to complete a short questionnaire which will assess your level of nicotine dependence, a saliva test will be used to confirm smoking status, and a blood and urine sample will be taken to see how well your kidneys are working. At each following visit, you will be asked to complete a set of other questionnaires that ask you about your craving for cigarettes, signs of withdrawal from smoking, your current mood and any side effects you may have experienced. At each visit, there will always be someone to help you if you have any queries or problems in completing the questionnaires. Blood and urine samples will be taken. A saliva test will be used to check your smoking status.

Summary of visits to the School of Pharmacy:

Visit	What will happen	Questions asked	How long will you be at the School of Pharmacy?
Screening	We will ask you some questions to check if you can join the study. A blood sample and urine dipstick test will be taken. If you are eligible you will be asked to sign a written consent form	Questions to determine • your level of nicotine dependence • how satisfying you currently find smoking	1 hour
Day 1	A saliva test will be used to check your smoking status. A blood sample will be taken just before you take the pill, then 2, 4, 6,	Questions to determine • how satisfying you currently find smoking • any signs of withdrawal from smoking	10 hours

	8, 10 hours later. Your blood pressure, pulse and heart rate will be measured just before you take the pill, then at 0, 2, 4, 8, 10 hours. We will also need to take a sample of urine from you.	• mood (asked at 0, 2, 4, 8, 10 hours) • how much you have an urge to smoke (asked at 0, 2, 4, 6 and 8,10 hours.	
Day 2	A saliva test will be used to check your smoking status. A single blood and urine sample will be collected from you, and your blood pressure, pulse and breathing rate will be measured	Questions to determine • how satisfying you currently find smoking • any signs of withdrawal from smoking • mood • how much you have an urge to smoke	1 hour
Day 3	As for day 2	As for day 2	1 hour
Day 4	As for day 2	As for day 2	1 hour
Day 5	As for day 2	As for day 2	1 hour
Day 6	As for day 2	As for day 2	1 hour
Day 13	As for day 2	As for day 2	1 hour
Day 14	As for day 2	As for day 2	1 hour
Day 17	As for day 2	As for day 2	1 hour
Day 18	As for day 2	As for day 2	1 hour
Day 20	As for day 2	As for day 2	1 hour
Day 21	As for day 2	As for day 2	1 hour
Day 25	A saliva test will be used to check your smoking status. A blood sample will be taken just before you take the pill, then 2, 4, 6 hours later. Your blood pressure, pulse and heart rate will be measured just before you take the pill, then at 0, 2, 4, 6 hours. We will also need to take a sample of urine from you.	Questions to determine • how satisfying you currently find smoking • any signs of withdrawal from smoking • mood (asked at 0, 2, 4, 6 hours) how much you have an urge to smoke (asked at 0, 2, 4, and 6 hours.	6 hours
Day 26	A single blood and urine sample will be collected from you, and your blood pressure, pulse and breathing rate will be measured	Questions to determine • how satisfying you currently find smoking • any signs of withdrawal from smoking • mood • how much you have an urge to smoke	1 hour

Will there be any costs involved?
The study will require you to spend one day at the School of Pharmacy and to visit the School on the following day and possibly the day after. You will be provided with petrol vouchers to compensate you for any travel expenses and you will be offered a reimbursement of up to $400 for your time. This will be paid at the end of your time in the study

What are the risks and benefits of this study?
Possible benefits
You will have the opportunity to try and quit smoking as part of this study. You will also have your blood pressure monitored. At the end of the study we will be able to refer you for further support for quitting smoking if required.

Possible risks

Cytisine is a medicine derived from a plant; *Cytisus laburnum* (Golden Rain) which has been used in Europe for over 40 years as a smoking cessation product. It does not contain any nicotine, but is similar in structure to nicotine. It is currently not available for sale in New Zealand. If you take more than the recommended dose of cytisine you may experience nausea (feeling sick), vomiting, pupil dilation, a racing heart rate and general weakness. These feelings will disappear if you reduce the amount of cytisine you are taking to the recommended levels. Cytisine should not to be taken by women who are pregnant or breastfeeding. It is considered safe and does not affect your ability to drive or operate machinery. The risks associated with taking blood from your arm are small, and may include bleeding, fainting, light-headedness, bruising, and/or an infection.

Your GP will be contacted if we find any abnormal results from your blood, urine or blood pressure tests, or in the unlikely event you have any negative reaction to the medicine.

Blood and urine samples
Blood and urine samples will be taken at screening to determine your kidney function. They will also be taken on study days to analyse what the body does to the drug once it is taken by you. All samples will be handled at the Auckland University.

If you wish to have any unused (excess) samples returned to you, you can indicate this on the consent form. Otherwise, blood and urine samples will be destroyed after analysis – the blood will be sterilized using steam, then buried. The urine samples will be flushed away).

Compensation
In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by the Accident Compensation Corporation (ACC) legislation. ACC cover is not automatic and each case is assessed by ACC, according to the provisions of the 2001 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are a wage earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, please contact your nearest ACC office or ask us for more information before you agree to take part.

Will the information about me be kept confidential?
The study files and all information that you provide will remain strictly confidential (private). No material that could personally identify you will be used in any reports on this study. The information will be kept securely at the School of Pharmacy, The University of Auckland and destroyed after 15 years according to national research guidelines. All computer records will be password protected. All future use of the information collected will be strictly controlled in accordance with the Privacy Act, 1994. During the study, ethics committee representatives, study personnel, and members of the research team may review the information collected. This will only be done to check the accuracy of the information collected for the study and the information will remain confidential.

When will the results be available?
This study may take six months to run, so results may not be available until 2012. You will be asked if you would like to be sent a copy of the results of the study when you agree to participate.

Has the study received ethical approval?
Yes, this study has received ethical approval from the Northern X Regional Ethics Committee, which reviews Regional studies. Ethics reference number: NTX/11/05/038

What are my legal rights?
Your participation in this study is entirely voluntary (your choice). You do not have to take part. If you choose not to take part in this study you or your future healthcare will not be affected in any way. You may withdraw from the study at any time, without having to give a reason. Your withdrawal from the study will not affect your future health care from the smoking cessation programme or any other health service. You are encouraged to ask questions at any time during the study. If you have any questions please ask a study research assistant or contact:

Associate Professor Janie Sheridan,
Principle Investigator,
School of Pharmacy,

Faculty of Medical and Health Sciences,
The University of Auckland,
Private Bag 92019,
Auckland.
Ph: (09) 373 7599 extension 85247
Fax: (09) 367 7192
Email: j.sheridan@auckland.ac.nz

If you have any questions or concerns regarding your rights as a participant in this study you may wish to contact a Health and Disability Advocate at the Health Advocates Trust:

Northland to Franklin ph 0800 555 050

Study Investigators at the Faculty of Medical and Health Sciences, University of Auckland

- Associate Professor Janie Sheridan, School of Pharmacy
- Associate Professor Malcolm Tingle, Department of Pharmacology and Clinical Pharmacology
- Dr David Newcombe, School of Population Health
- Dr Natalie Walker, School of Population Health
- Soo Hee Jeong

Please keep this sheet for your information.
Thank you for taking the time to read about this study.

Appendix 4 Phone screening

The steps and questions that were asked during the phone screening are outlined below.

Screening phone call

A. Before you can participate, I need to assess whether you are able to join the study. I will be asking you a series of questions which may take roughly 15 minutes.

DEMOGRAPHIC DETAILS:

1. **Could you tell me your age?**
   a. *18 or older: Move onto next section*
   b. *Under 18: I'm sorry, but unfortunately, you are only eligible to participate in the study if you are 18 years of age or older. Thank you for time.*

B. Exclusion Criteria:

1. Females: **Are you pregnant or breastfeeding?**
   a. *No: Move onto next question*
   b. *Yes: I'm sorry, but unfortunately, because you are pregnant/breastfeeding, you are not eligible to take part in the study. Thank you for your time.*
2. **Have you had a. Stroke, b. Heart attack or c. Severe angina in the last two weeks?**
   a. *No: Move onto next question*
   b. *Yes: I'm sorry, but unfortunately, because you have had the following conditions (…) in the last 2 weeks, you are not eligible to take part in the study. Thank you for your time.*
3. **Have you been diagnosed with severe/uncontrolled hypertension? (>150/>100)**
   a. *No: Move onto next question*
   b. *Yes: I'm sorry, but unfortunately, because you have severe/uncontrolled hypertension, you are not eligible to take part in the study. Thank you for your time.*
4. **Have you been diagnosed with schizophrenia?**
   a. *No: Move onto next question*
   b. *Yes: I'm sorry, but unfortunately, because you have been diagnosed with schizophrenia, you are not eligible to take part in the study. Thank you for your time.*

Page 1 of 3

5. **Have you been diagnosed with phaeochromocytoma?**
    a. *No: Move onto next question*
    b. *Yes: I'm sorry, but unfortunately, because you have been diagnosed with phaeochromocytoma, you are not eligible to take part in the study. Thank you for your time.*
6. **Do you have severe renal impairment?**
    a. *No: Move onto next question*
    b. *Yes: I'm sorry, but unfortunately, because you have severe renal impairment, you are not eligible to take part in the study. Thank you for your time.*
7. **Do you only smoke non-cigarette tobacco products? (e.g. pipes, cigars)**
    a. *No: Move onto next question*
    b. *Yes: I'm sorry, but unfortunately, because you are only using non-cigarette tobacco products, you are not eligible to take part in the study. Thank you for your time.*
8. **Are you currently using nicotine replacement products? (e.g patches, gum, lozenges)**
    a. *No: Move onto next question*
    b. *Yes: I'm sorry, but unfortunately, because you are currently using NRT products, you are not eligible to take part in the study. Thank you for your time.*
9. **Are you currently using any non-nicotine-based medicines such as A. Zyban (bupropion), B. Catapres (clonidine), C. Norpess (notriptyline) or D. Champix (varenicline)**
    a. *No: Move onto next question*
    b. *Yes: I'm sorry, but unfortunately, because you are using other non-nicotine based medicines that can help you quit smoking, you are not eligible to take part in the study. Thank you for your time.*
10. **Are you enrolled in another smoking cessation programme or another cessation study?**
    a. *No: Move onto next section*
    b. *Yes: I'm sorry, but unfortunately, because you are taking part in another cessation programme/study you are not eligible to take part in the study. Thank you for your time.*

C. Great, now I'm going to ask you some questions to determine your nicotine dependence. (administer FTND)

1. How soon after you wake up do you smoke your first cigarette?
2. Etc.
    a. *HSI score higher than 2: move onto next question*
    b. *HSI score less than 2: I'm sorry, but unfortunately, your nicotine dependence score is lower than what is required to take part in the study. Thank you for your time.*

D. Great, so based on what you have answered, it appears that you may be able to take part in our study so you will need to come in for a short screening visit where you will be asked to:

    a. Fill out your personal details (name, phone number etc)
    b. Sign a consent form
    c. Have your vital signs measured (blood pressure, pulse and breathing rate)
    d. Take a small saliva sample to confirm your smoking status
    e. Take a small blood and urine sample to determine your renal function

This will take no more than an hour. When would be a good time for you? (Arrange time)

E. Any questions about the study? (Refer to PIS)

F. Thank participant. Confirm meeting date/time for screening visit.

Appendix 5 Questionnaires

Fagerström Test for Nicotine Dependence (FTND)

1. How soon after you wake up do you smoke your first cigarette?

a. Within 5 minutes	3 points
b. 6-30 minutes	2 points
c. 30-60 minutes	1 point
d. After 60 minutes	0 points

2. Do you find it difficult to refrain from smoking in places where it is forbidden, e.g., in church, at the library, in the cinema, etc.?

a. Yes	1 point
b. No	0 points

3. Which cigarette would you hate most give up?

a. The first one in the morning	1 point
b. Any other	0 points

4. How many cigarettes/day do you smoke?

a. 10 or less	0 points
b. 11-20	1 point
c. 21-30	2 points
d. 31 or more	3 points

5. Do you smoke more frequently during the first hours after awakening than during the rest of the day?

a. Yes	1 point
b. No	0 points

6. Do you smoke if you are so ill that you are in bed most of the day?

a. Yes	1 point
b. No	0 points

Modified Cigarette Evaluation Questionnaire (mCEQ)

If you have smoked since you last completed this questionnaire, please mark the number that best represents how smoking made you feel (1- not at all, 2- vey little, 3- a little, 4- moderately, 5- a lot, 6- quite a lot, 7- extremely).

1. Was smoking satisfying?	1	2	3	4	5	6	7
2. Did cigarettes taste good?	1	2	3	4	5	6	7
3. Did you enjoy the sensations in your throat and chest?	1	2	3	4	5	6	7
4. Did smoking calm you down?	1	2	3	4	5	6	7
5. Did smoking make you feel more awake?	1	2	3	4	5	6	7
6. Did smoking make you feel less irritable?	1	2	3	4	5	6	7
7. Did smoking help you concentrate?	1	2	3	4	5	6	7
8. Did smoking reduce your hunger for food?	1	2	3	4	5	6	7
9. Did smoking make you dizzy?	1	2	3	4	5	6	7
10. Did smoking make you nauseous?	1	2	3	4	5	6	7
11. Did smoking immediately relieve your craving for a cigarette?	1	2	3	4	5	6	7
12. Did you enjoy smoking?	1	2	3	4	5	6	7

Mood and Physical Symptoms Scale (MPSS)

Please show for each of the items how you have been feeling over the past 24 h (circle one number for each item).

	Not at all	Slightly	Somewhat	Very	Extremely
Depressed	1	2	3	4	5
Irritable	1	2	3	4	5
Restless	1	2	3	4	5
Hungry	1	2	3	4	5
Poor concentration	1	2	3	4	5

How much of the time have you felt the urge to smoke in the past 24 h? (circle one number).

All the time	Almost all the time	A lot of the time	Some of the time	A little of the time	Not at all
5	4	3	2	1	0

How strong have the urges been? (circle one number).

Extremely strong	Very strong	Strong	Moderate	Slight	No urges
5	4	3	2	1	0

Questionnaire on smoking urges-brief (QSU-brief)

Please circle one (1=strongly disagree to 7=strongly agree)

1. I have a desire for a cigarette right now.	1	2	3	4	5	6	7
2. Nothing would be better than smoking a cigarette right now.	1	2	3	4	5	6	7
3. If it were possible I would probably smoke now.	1	2	3	4	5	6	7
4. I could control things better right now if I could smoke.	1	2	3	4	5	6	7
5. All I want right now is a cigarette.	1	2	3	4	5	6	7
6. I have an urge for a cigarette.	1	2	3	4	5	6	7
7. A cigarette would taste good now.	1	2	3	4	5	6	7
8. I would do almost anything for a cigarette now.	1	2	3	4	5	6	7
9. Smoking would make me less depressed.	1	2	3	4	5	6	7
10. I am going to smoke as soon as possible.	1	2	3	4	5	6	7

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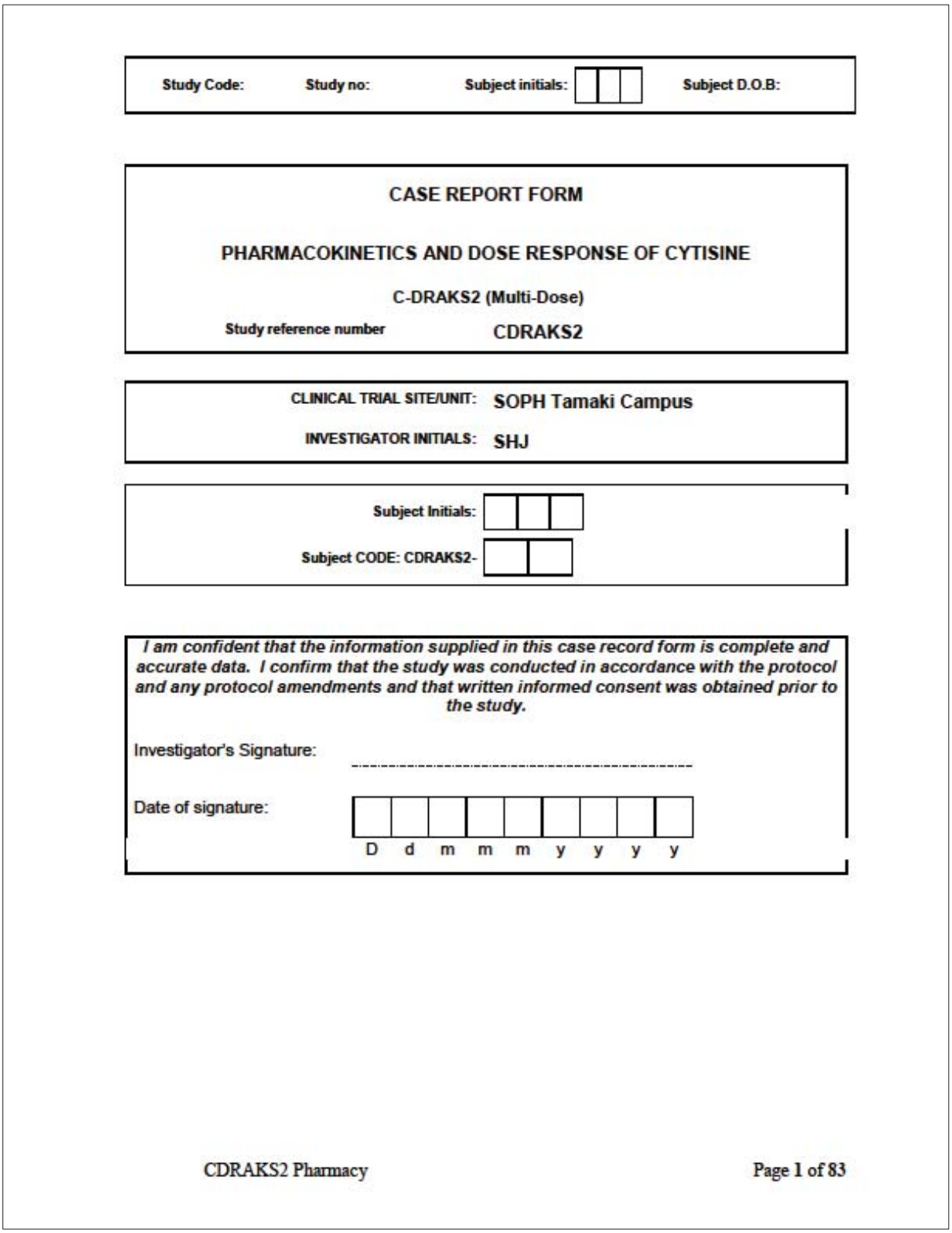
Appendix 6 Case Report Forms (CRFs)

Example pages of the CRF (multi-dose study) are provided.



Study Code: Study no: Subject initials: Subject D.O.B:

CASE REPORT FORM

PHARMACOKINETICS AND DOSE RESPONSE OF CYTISINE

C-DRAKS2 (Multi-Dose)

Study reference number CDRAKS2

CLINICAL TRIAL SITE/UNIT: SOPH Tamaki Campus

INVESTIGATOR INITIALS: SHJ

Subject Initials:

Subject CODE: CDRAKS2-

I am confident that the information supplied in this case record form is complete and accurate data. I confirm that the study was conducted in accordance with the protocol and any protocol amendments and that written informed consent was obtained prior to the study.

Investigator's Signature: _____

Date of signature:

D d m m m y y y y

CDRAKS2 Pharmacy Page 1 of 83

Study Code:	Study no:	Subject initials:		Subject D.O.B:

SCREENING

Date: _____
DD MM YYYY

	Yes	No*
Inclusion Criteria		
1. Subject is 18 years of age or older	☐	☐
2. Subject has read and understood the Participant Information sheet.	☐	☐
3. Subject has provided a written consent.	☐	☐
4. Subject has moderate/heavy nicotine dependence (determined by FTND).	☐	☐

**If any inclusion criteria are ticked no then the patient is not eligible for the study.*

	Yes*	No
Exclusion Criteria		
1. Subject is pregnant or breastfeeding.	☐	☐
2. Subject has had the following condition in the last two weeks:		
a. Stroke	☐	☐
b. Heart attack	☐	☐
c. Severe angina	☐	☐
3. Subject is diagnosed with severe/uncontrolled hypertension. (systolic blood pressure greater than 150mmHg, diastolic greater than 100mmHg)	☐	☐
4. Subject is diagnosed with schizophrenia.	☐	☐
5. Subject is diagnosed with phaeochromocytoma.	☐	☐
6. Subject has severe renal impairment.	☐	☐
7. Subject only smokes non-cigarette tobacco products (e.g. pipes, cigars)	☐	☐
8. Subject is a current user of Nicotine Replacement Therapy (NRT) products (e.g. patches, gum, lozenges).	☐	☐
9. Subject is currently using non nicotine-based medicines such as:		
a. Zyban (bupropion)	☐	☐
b. Catapres (clonidine)	☐	☐
c. Norpess (nortriptyline)	☐	☐
d. Champix (varenicline)	☐	☐
10. Subject is enrolled in another smoking cessation programme or another cessation study.	☐	☐

**If any inclusion criteria are ticked yes then the patient is not eligible for the study.*

Study Code:	Study no:	Subject initials:		Subject D.O.B:

SCREENING

Date: _____
DD MM YYYY

INFORMED CONSENT

Please note: written informed consent must be given before any study specific procedures take place or any current therapy is discontinued for the purposes of participation in this study.

Has the subject freely given written informed consent? Yes ☐ No ☐

DEMOGRAPHIC DATA

Age (yrs): ☐☐ Sex: Female ☐ Male ☐

SMOKING STATUS

NicAlert Nicotine Test result: Level ☐

Above cut-off point (100ng/mL))? Yes ☐ No ☐

Smoking history (number of years): ☐☐

How many cigarettes per day? ☐☐

Fagerstrom score (out of 10): ☐☐

Previous quit attempts? ☐☐

MEDICATIONS TAKEN

Is the subject currently or previously taking any medication including OTC, vitamins and/or supplements? Yes ☐ No ☐

*Record **all** medication on Concomitant Medications page

Study Code:	Study no:	Subject initials:	Subject D.O.B:

SCREENING

VITAL SIGNS

	1	2	3	Mean
Heart rate (Bpm)				
Blood pressure (seated) DBP/SBP (mmHg)	/	/	/	/
Breathing rate (breaths per minute)				

RENAL FUNCTION

Dipstick test results:
Leukocytes _____
Nitrite _____
Protein _____
Glucose _____
Blood _____

Blood test:
Creatinine level: _____ umol/L (Ref 60-105
EGFR _____ (Ref >90)

Renal function acceptable: Yes ☐ No ☐

*If *NO* for above, the participant is not eligible for the study.

Study Code:	Study no:	Subject initials:		Subject D.O.B:

SCREENING

End of Visit Checklist: to be completed by Investigator

		Yes	No
1	Does the subject satisfy the inclusion and exclusion criteria to date?	☐	☐
2	Have all screening procedures been completed?	☐	☐
3	Has the concomitant medication page been completed?	☐	☐
4	Is the subject willing to proceed?	☐	☐

Investigator

	Yes	No
Is the subject to continue?	☐	☐
Have the dosing instructions been explained to the patient?	☐	☐
Reminder texts required?	☐	☐

STUDY DIARY REMINDER TEXTS @ _____ pm

Signature: ..... Date: ☐☐☐☐☐☐☐☐
d d m m y y y y

If 'Yes' please:

Complete details of next visit and any other needed instructions on the instruction card.

Give the subject the instruction card and cytisine leaflet.

Study Code:	Study no:	Subject initials:	Subject D.O.B:

DAY1: VITAL SIGNS

At T=0h

	1	2	3	Mean
Heart rate (Bpm)				
Blood pressure (seated) DBP/SBP (mmHg)	/	/	/	/
Breathing rate (breaths per minute)				

At T=2h (hours post dose)

	1	2	3	Mean
Heart rate (Bpm)				
Blood pressure (seated) DBP/SBP (mmHg)	/	/	/	/
Breathing rate (breaths per minute)				

At T=4h (hours post dose)

	1	2	3	Mean
Heart rate (Bpm)				
Blood pressure (seated) DBP/SBP (mmHg)	/	/	/	/
Breathing rate (breaths per minute)				

Study Code:	Study no:	Subject initials:	Subject D.O.B:

At T=8h (hours post dose)	1	2	3	Mean
Heart rate (Bpm)				
Blood pressure (seated) DBP/SBP (mmHg)	/	/	/	/
Breathing rate (breaths per minute)				

At T=10h (hours post dose)	1	2	3	Mean
Heart rate (Bpm)				
Blood pressure (seated) DBP/SBP (mmHg)	/	/	/	/
Breathing rate (breaths per minute)				

BLOODS

Sample	Time	Hours post dosing	Amount collected (mL)
1		0	
2		2	
3		4	
4		6	
5		8	
6		10	

URINE

Sample	Time	Hours post dosing	Amount collected (mL)
1			
2			
3			
4			
5			
6			

Study Code: Study no: Subject Initials: Subject D.O.B:

CONCOMITANT MEDICATIONS

Medication	Total Daily Dose	Units	Reason	Start Date (MM/DD/YYYY)	Stop Date (MM/DD/YYYY)	Continue?
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐
				__/__/____	__/__/____	☐

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Study Code:	Study no:	Subject initials:		Subject D.O.B:

OFF STUDY FORM

Date Off Study: ___/___/_____
(MM/DD/YYYY)

Date Last Study Medication Taken: ___/___/_____
(MM/DD/YYYY)

Reason Off Study (Please mark only the primary reason. Reasons **other than Completed Study** require explanation next to the response)

- ☐ Completed study
- ☐ AE/SAE **(complete AE CRF & SAE form, if applicable)** _____
- ☐ Lost to follow-up _____
- ☐ Non-compliant participant _____
- ☐ Concomitant medication _____
- ☐ Medical contraindication _____
- ☐ Withdraw consent _____
- ☐ Death **(complete SAE form)** _____
- ☐ Other _____

Questions on Medications

Study code		Subject initials				D.O.B	

Do you take medicines for any of the following:

		If yes, please name the medication
1. Asthma, allergies, hay fever, or sinusitis?	___ yes ___ no	
2. Heart problems	___ yes ___ no	
3. Diabetes or thyroid problems?	___ yes ___ no	
4. Liver, kidney, or lung disease?	___ yes ___ no	
5. Ulcers or stomach problems?	___ yes ___ no	
6. Hepatitis or jaundice?	___ yes ___ no	
7. Epilepsy or strokes?	___ yes ___ no	
8. High blood pressure	___ yes ___ no	
9. Bleeding or clotting disorders?	___ yes ___ no	
10. Arthritis, painful joints, or skin rash?	___ yes ___ no	
11. Cancer,	___ yes ___ no	
12. AIDS	___ yes ___ no	
13. Depression or anxiety	___ yes ___ no	
14. Schizophrenia/schizoaffective disorder	___ yes ___ no	
15. Chronic pain	___ yes ___ no	
16. Any other medicines taken not mentioned above	___ yes ___ no	

Appendix 7 Study Diary

All participants in the multi-dose study were provided with a study diary to record sleep, dosing and smoking for each day during the study (Days 1 – 26). Participants were also asked to note down any unusual occurrences (side-effects) in the comments section. Example pages of the study diary are provided.

Drug information

Trade name: Tabex

Active ingredient: Cytisine

Drug form: Filmtablets of 1.5 mg

Composition: One filmtablet contains (mg)
Active substance: Cytisine 1.5mg
Other ingredients: Calcium dihydrogenphosphate, Lactose, Wheat starch, Microcrystalline cellulose, Talc, Magnesium stearate.

Pharmaco-therapeutic group: Vegetotropic agent - N- cholinomimetic (gangliostimulant).

Indications: Chronic nicotinism (tabacosis) - for breaking the habit of smoking.

Contraindications: Tabex is contraindicated in arterial hypertension and advanced atherosclerosis.

Warning: The clinical studies with Tabex show that the Bulgarian drug is effective and well tolerated. The adverse effects are insignificant in case of adequate treatment according to the schedule and do not necessitate reduction of the dose and shortening the duration of the treatment. The physicians should warn the patients that the simultaneous administration of the drug and smoking could lead to aggravated adverse effects of nicotine (nicotine intoxication). The drug should be used in all cases when the patient has a honest and firm intention to give up smoking.

There is not sufficient clinical experience with Tabex administration to patients with ischemic heart disease, cardiac impairment, cerebrovascular diseases, obliterating arterial diseases, hyperthyroidism, diabetes mellitus, renal and hepatic insufficiency. The use of the drug of these categories of patients should be performed only after the potential benefit has been weighed against the possible risks.

Pregnancy and lactation: The intraovular application of Cytisine substance to hen embryos induced no embriotoxic and teratogenic effect within the limits of the single therapeutic doses. Higher doses of the drug lead to embryotoxic action. On the base of the experimental data obtained, Tabex is recommended (with Cytisine as the basic component) not to be taken by pregnant women, due to the potential risk of embryotoxic action in case of uncontrolled administration. The drug should not be administered during breast feeding.

Effect of the drug on drivers and machine operators: Tabex is considered safe and induces no changes in the physchophysical status, driving ability and machine operation.

Drug interactions: No data is available on undesired interactions between Tabex and other pharmaceuticals.

Overdose: Symptoms of nicotine intoxication are observed in Tabex overdose. The toxic effects are manifested in nausea, vomiting, pupil dilation, tachycardia, general weakness, clonic convulsions, paralysis of respiration. The communications of overdose with the drug are scarce. Lavage of the stomach, monitoring of respiration, arterial pressure and heart rate are initiated as in all cases of overdose. Infusion, reanimation is undertaken with saline and glucose solutions, anticonvulsants, cardiotonics, analeptics, etc. symptomatic agents.

Administration and dosage: The drug is administered perorally according to the following schedule:

- First 3 days: 1 tablet 6 times daily (every 2 hours) with a parallel reduction of the number of cigarettes smoked.
- 4th to 12th day: 1 tablet every 2 1/2 hours (5 tablets daily)
- 13th to 16th day: 1 tablet every 3 hours (4 tablets daily)
- 17th to 20th day: 1 tablet every 5 hours (3 tablets daily)
- 21st to 25th day: 1 to 2 tablets daily

Smoking cessation should occur by the 5th day after the initiation of the treatment. After the end of the therapeutic course, in order to have good results, the patient should give evidence of strong will, not allowing the lighting of a cigarette.

Undesired adverse effects: The clinical studies showed a good tolerance to the drug and grave adverse effects were not observed. The following adverse effects are rather often observed at the beginning of Tabex treatment: changes in both taste and appetite, dryness in the mouth, headache, irritability, nausea, constipation, tachycardia, light elevation of the arterial pressure. The majority of the adverse effects can abate in the course of the treatment.

Supplied: The filmtablets Tabex are packed in PVC/aluminum foil blister strips. Each blister strip contains 20 filmtablets. Five blister strips are packed in a cardboard box together with package insert.

Storage: In original packages, in dry, and light-protected premises at temperature of 15° -25° C (60° - 75° F).

Expiry term: Two year from manufacture date.

How dispensed: No physician prescription required.

Name and address of manufacturer: Sopharma AD, 16 Iliensko Chossee St. Sofia, Bulgaria 1220 (www.sopharma.com)

Appointments

Visit (Study Day)	*When?*	*For how long?*
1 (Day 1)		10 hours
2 (Day 2)		1 hour
3 (Day 3)		1 hour
4 (Day 4)		1 hour
5 (Day 5)		1 hour
6 (Day 6)		1 hour
7 (Day 13)		1 hour
8 (Day 14)		1 hour
9 (Day 17)		1 hour
10 (Day 18)		1 hour
11 (Day 21)		1 hour
12 (Day 22)		1 hour
13 (Day 25)		6 hours
14 (Day 26)		1 hour

Please remember to bring this diary and medication to the next clinic visit. At the end of the study please bring any unused study drugs and any empty containers.

Study Day 1 Date:_____

SLEEPING DIARY

1. Last night I went to bed at: ____am/pm.	
2. After turning out the lights, I fell asleep in ___ minutes.	
3. This morning I woke up at ____am/pm.	
4. I slept a total of ___ hours last night.	
5. My sleep was interrupted ___ times. Specify the number of night-time awakenings.	
6. When I got up this morning, I felt: Please rate on scale 1 to 5 (1=Exhausted, 5=Refreshed)	
7. Overall, my sleep last night was: Please rate on scale 1 to 5 (1=Very restless, 5=Very sound)	

DOSING DIARY

Instructions: 1 tablet every 2h (6/day)			
	Time	Amount	Comments
Dose 1			
Dose 2			
Dose 3			
Dose 4			
Dose 5			
Dose 6			

SMOKING DIARY

I smoked my first cigarette ____ minutes after waking up.	
I smoked ____ cigarettes before noon.	
I smoked ____ cigarettes from noon to 6pm.	
I smoked ____ cigarettes from 6pm to bedtime.	
I smoked a total of ____ cigarettes today.	

COMMENTS:

QUIT DAY

Study Day 5 Date:_____

SLEEPING DIARY

29. Last night I went to bed at: ____am/pm.	
30. After turning out the lights, I fell asleep in ___ minutes.	
31. This morning I woke up at ____am/pm.	
32. I slept a total of ___ hours last night.	
33. My sleep was interrupted ___ times. Specify the number of night-time awakenings.	
34. When I got up this morning, I felt: Please rate on scale 1 to 5 (1=Exhausted, 5=Refreshed)	
35. Overall, my sleep last night was: Please rate on scale 1 to 5 (1=Very restless, 5=Very sound)	

DOSING DIARY

Instructions: 1 tablet every 2.5h (5/day)			
	Time	Amount	Comments
Dose 1			
Dose 2			
Dose 3			
Dose 4			
Dose 5			

School of Pharmacy
University of Auckland

Study Day 25 Date:_____

SLEEPING DIARY

169. Last night I went to bed at: ____am/pm.	
170. After turning out the lights, I fell asleep in ___ minutes.	
171. This morning I woke up at ____am/pm.	
172. I slept a total of ___ hours last night.	
173. My sleep was interrupted ___ times. Specify the number of night-time awakenings.	
174. When I got up this morning, I felt: Please rate on scale 1 to 5 (1=Exhausted, 5=Refreshed)	
175. Overall, my sleep last night was: Please rate on scale 1 to 5 (1=Very restless, 5=Very sound)	

DOSING DIARY

Instructions: 1 tablet every 6h (2/day)			
	Time	Amount	Comments
Dose 1			
Dose 2			

SMOKING DIARY

I smoked my first cigarette ____ minutes after waking up.	
I smoked ____ cigarettes before noon.	
I smoked ____ cigarettes from noon to 6pm.	
I smoked ____ cigarettes from 6pm to bedtime.	
I smoked a total of ____ cigarettes today.	

COMMENTS:

END OF MEDICATION

Appendix 8 Adverse Events Form

Study Code: Study no: Subject Initials: Subject D.O.B:

CDRAKS2 ADVERSE EVENTS FORM

1. Detail of Adverse Event

	Specific diagnosis (Enter one diagnosis or signs/symptoms per line)	Onset date (DD/MM/YY)	Event type[1]	Maximum severity[2]	Frequency[3]	Outcome[4]	Relationship[5]	Report Type[6]	Withdrawn due to AE?	Date outcome status assessed (DD/MM/YY)
1.01									Yes ☐ No ☐	
1.02									Yes ☐ No ☐	
1.03									Yes ☐ No ☐	
1.04									Yes ☐ No ☐	

[1] **Event type**
0- Any serious event
1- Death
2- Life threatening
3- Hospitalisation
4- Persistent or significant disability
5- Congenital abnormality
6- Medically important

[2] **Maximum severity**
1- Mild
2- Moderate
3- Severe

[3] **Frequency**
1- Single episode
2- Intermittent
3- Continuous

[4] **Outcome**
1- Resolved
2- Ongoing
3- Death
4- Unknown/ lost to follow up

[5] **Relationship to study treatment**
1- Definitely
2- Probably
3- Possibly
4- Not related

[6] **Report Type**
1- Initial
2- Follow-up

CDRAKS2
AEs Form

Page 1 of 3

Study Code:	Study no:	Subject Initials:		Subject D.O.B:

2. Hospitalisation

	Yes	No	
2.01	☐	☐	Admitted to hospital (completed formal admission procedures)
			If Yes,
2.02			☐☐/☐☐/☐☐☐☐ Date admitted to hospital (DD/MM/YYYY)
2.03	☐	☐	Has left hospital?
			If Yes,
2.04			☐☐/☐☐/☐☐☐☐ Date discharged from hospital or date died in hospital (leave blank if still in hospital) (DD/MM/YYYY)

3. Death

	Yes	No	
3.01	☐	☐	Did the patient die?
			If Yes,
3.02			☐☐/☐☐/☐☐☐☐ Date of death (DD/MM/YYYY)
3.03			Proximate/immediate cause of death
3.04			Underlying cause of death

Study Code:	Study no:	Subject Initials:		Subject D.O.B:

4. Narrative/Comments

4.01 Narrative/comments (section to complete text description of Event)

5. Study Treatment

5.01 ☐☐/☐☐/☐☐☐☐ Date last dose of study treatment taken before the first event reported on this form
DD/MM/YYYY

6. Supporting Documentation

6.01 Is there a serious event (including a serious reaction to the study treatment) being reported?

Yes ☐ No ☐

If Yes

Yes ☐ No ☐ Supporting documentation is filed in participant's study folder?

7. Signature of Study Researcher

7.01 Signature _____ Printed name _____ ☐☐/☐☐/☐☐☐☐ DD/MM/YYYY

Appendix 9 Individual plasma cytisine concentrations measured in the multi-dose study

Participant 1

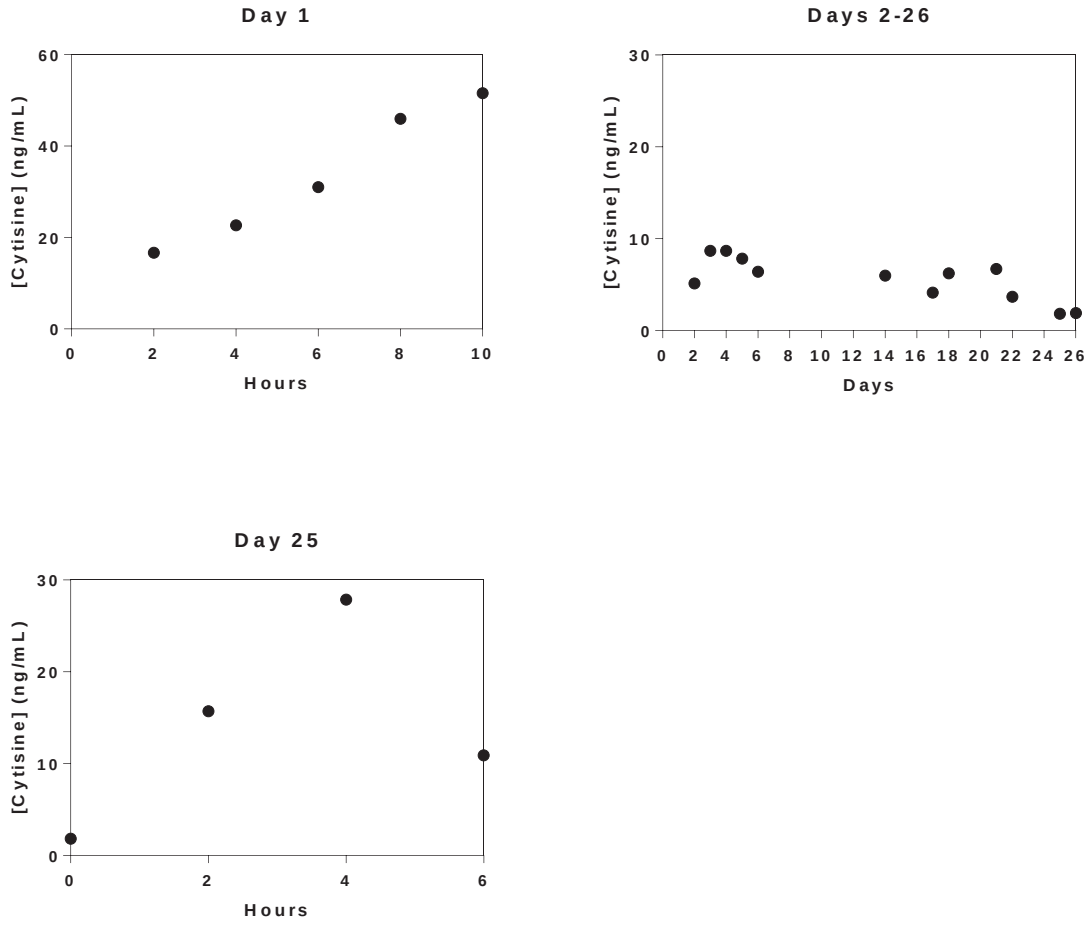

Figure 9.1. Trough plasma concentrations of cytisine measured in participant 1 during the study.

Participant 2

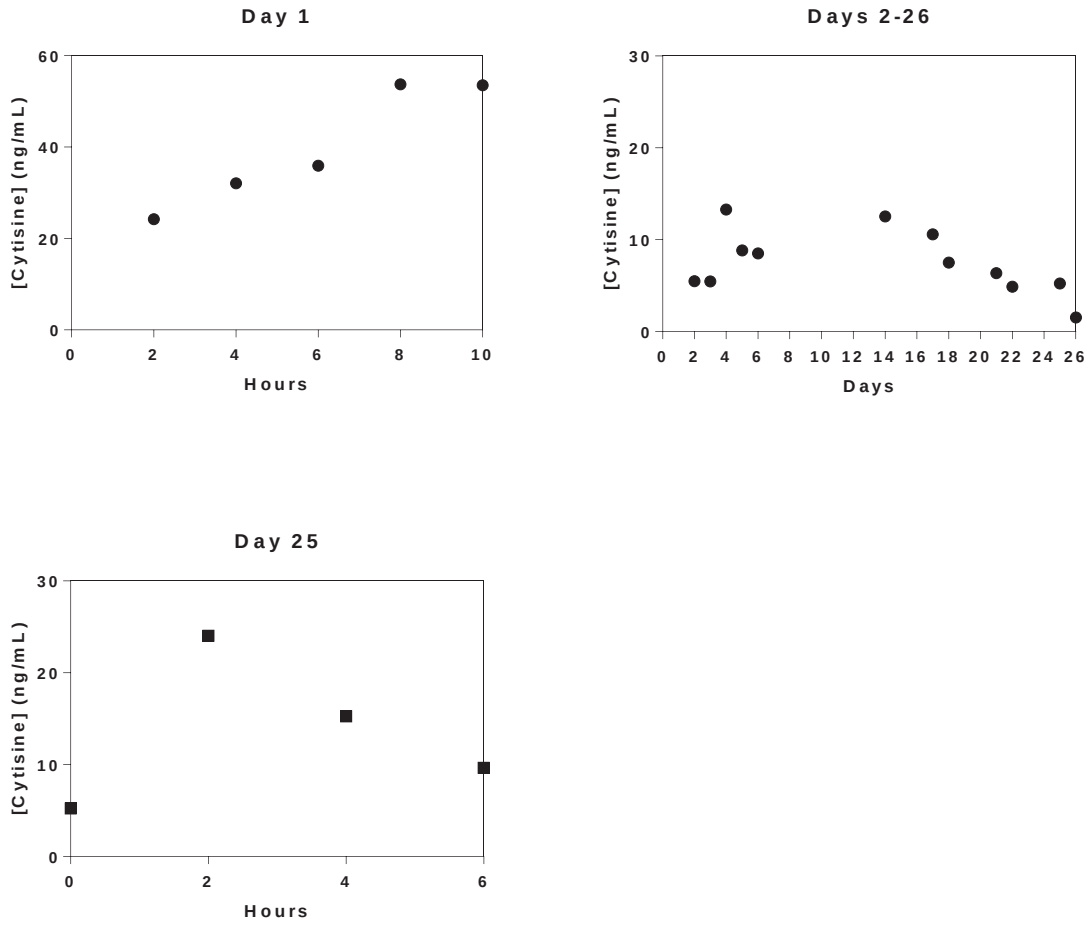

Figure 9.2: Trough plasma concentrations of cytisine measured in participant 2 during the study.

Participant 3

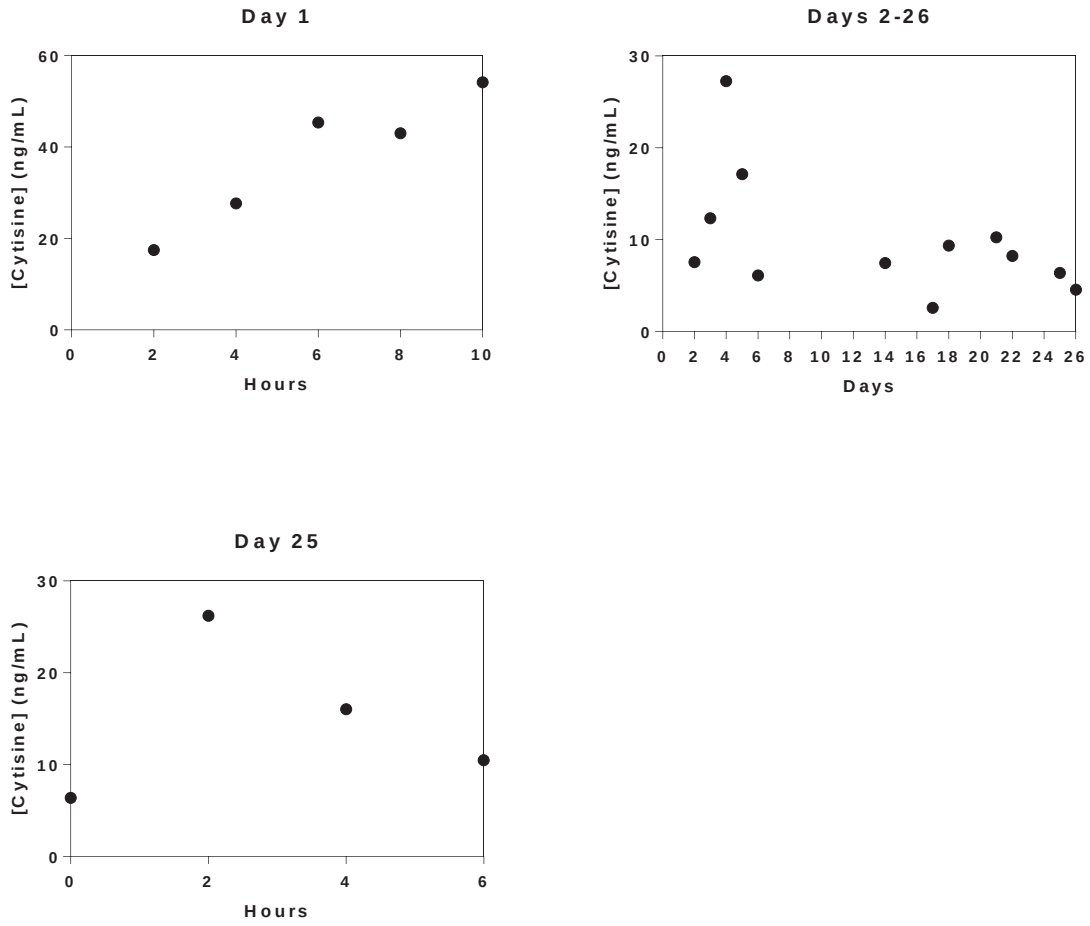

Figure 9.3: Trough plasma concentrations of cytisine measured in participant 3 during the study.

Participant 4

On day 14, the trough concentration measured in this subject appeared to be unusually high. Referring back to the dosing diary the subject filled out, the subject had reported to taken a tablet before coming in to provide a blood sample that morning.

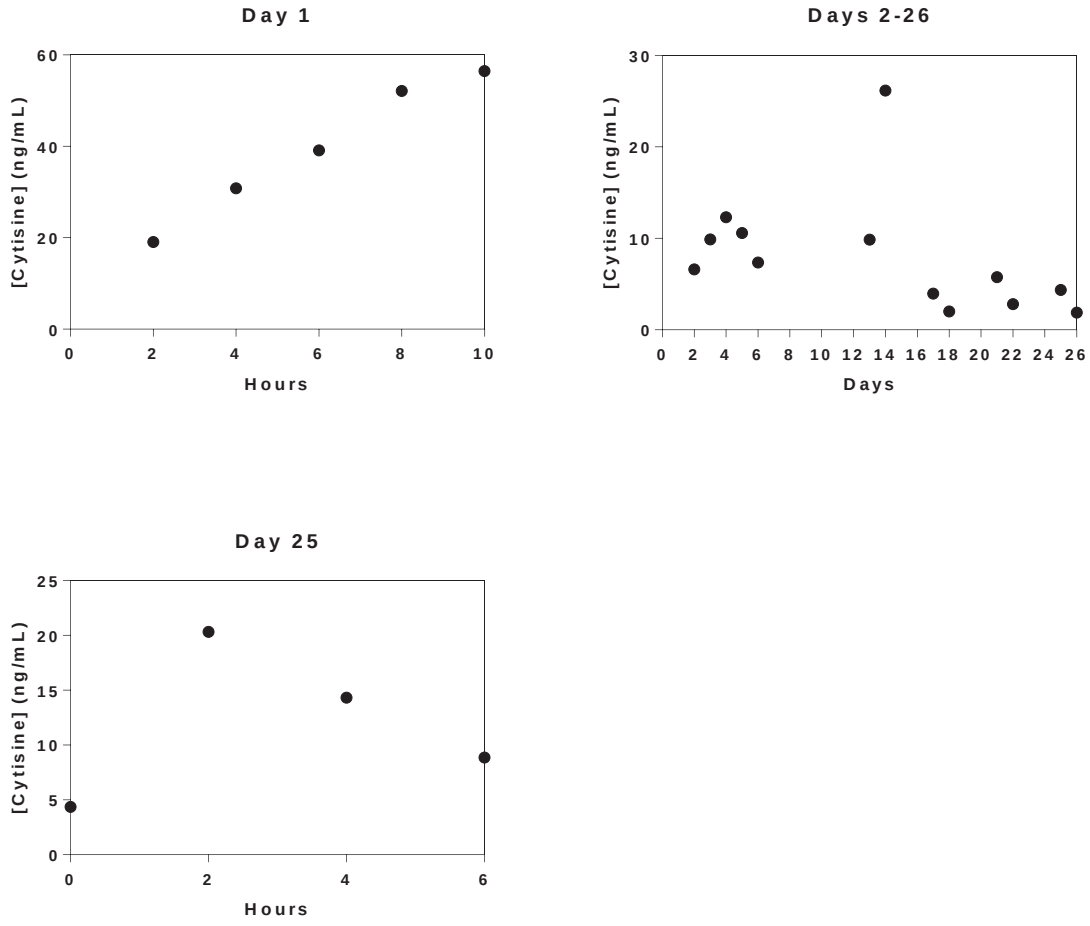

Figure 9.4: Trough plasma concentrations of cytisine measured in participant 4 during the study.

Participant 5

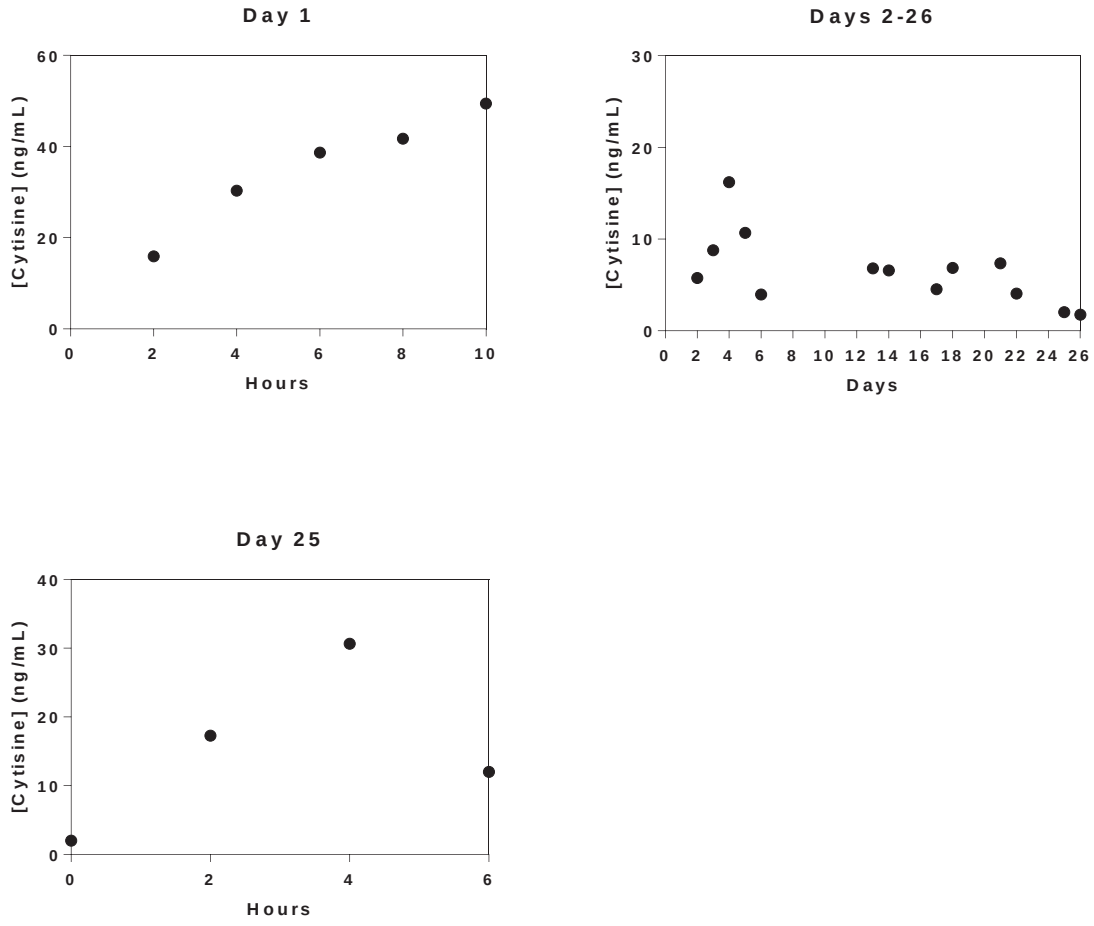

Figure 9.5: Trough plasma concentrations of cytisine measured in participant 5 during the study.

Participant 6

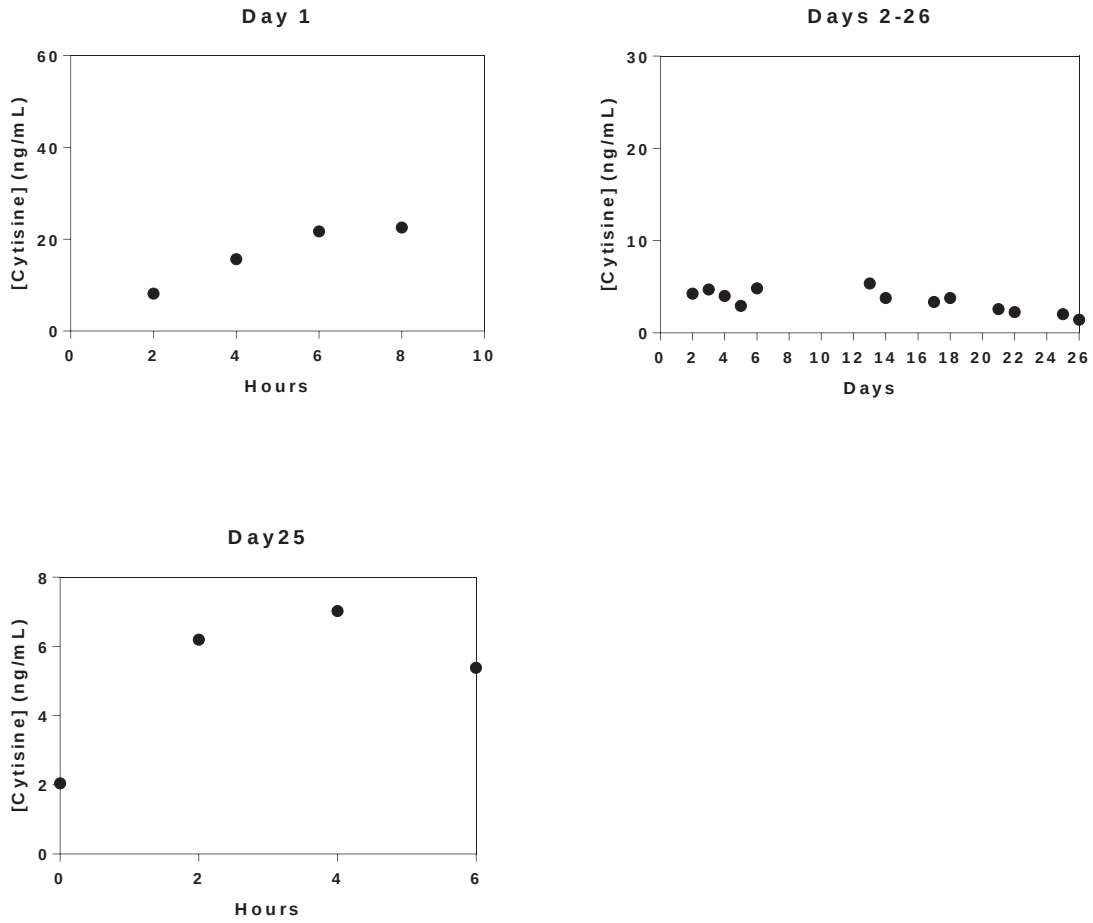

Figure 9.6: Trough plasma concentrations of cytisine measured in participant 6 during the study.

Participant 7

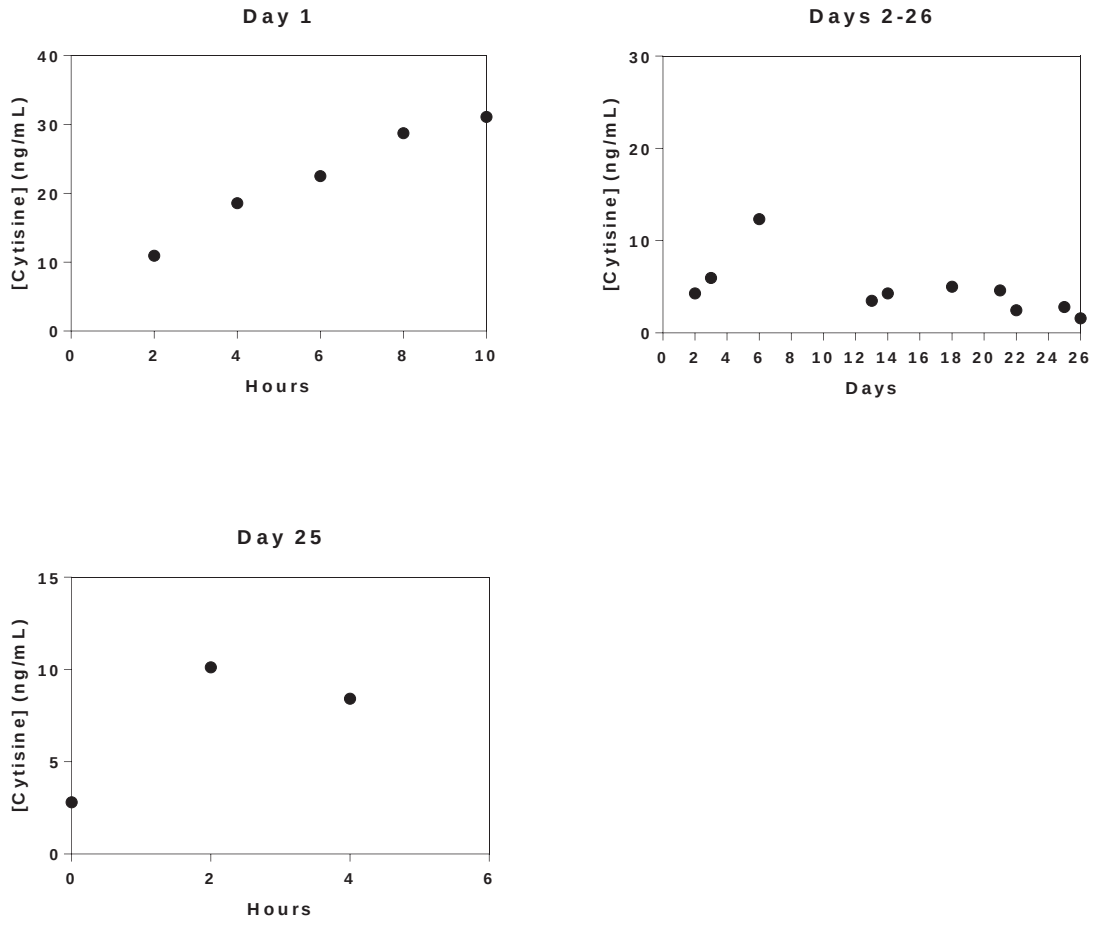

Figure 9.7: Trough plasma concentrations of cytisine measured in participant 7 during the study.

Participant 8

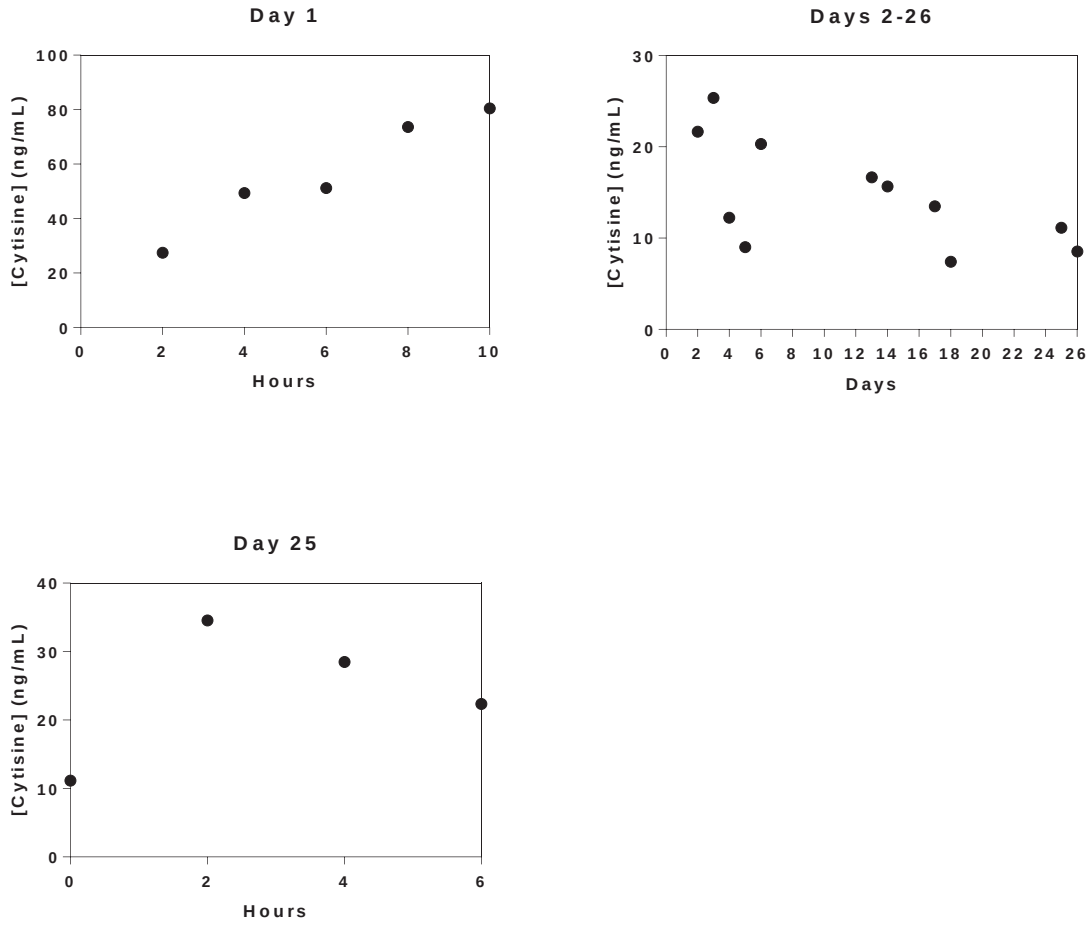

Figure 9.8: Trough plasma concentrations of cytisine measured in participant 8 during the study.

Participant 9

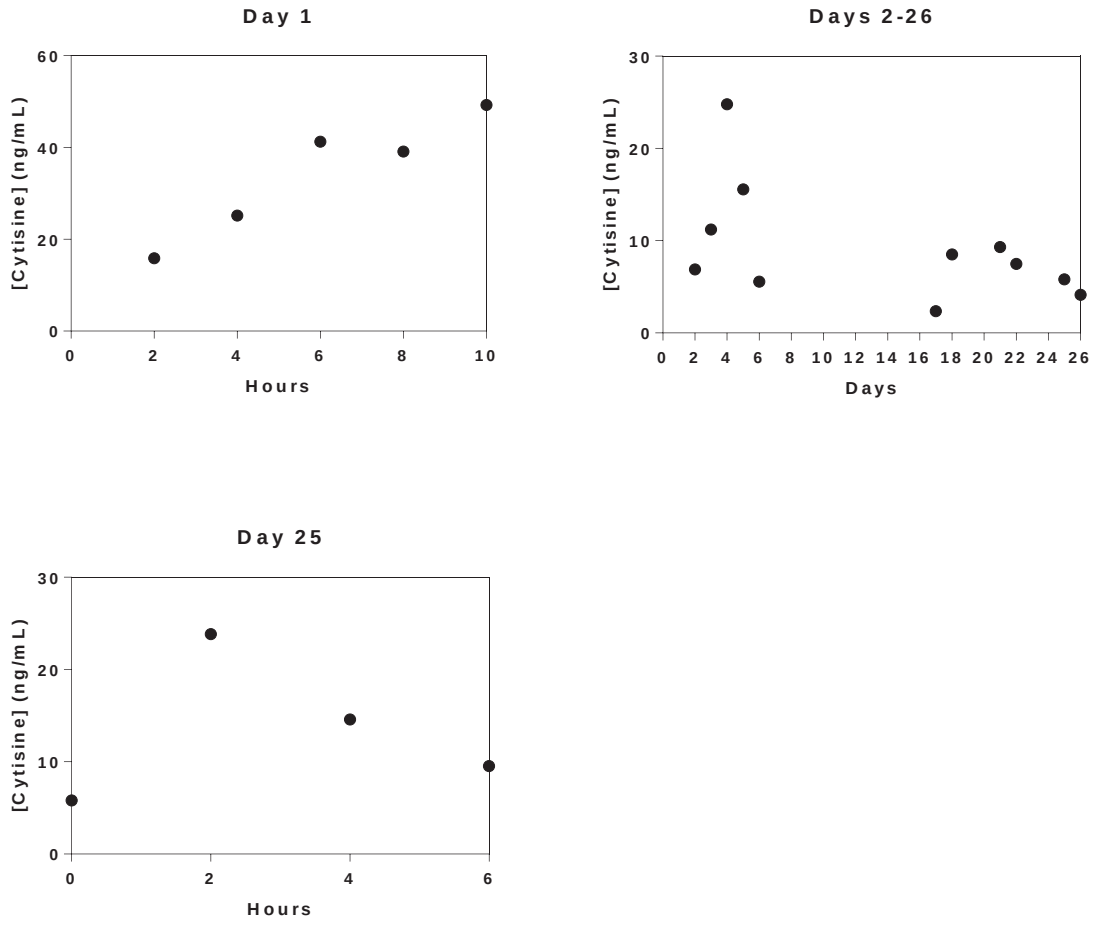

Figure 9.9: Trough plasma concentrations of cytisine measured in participant 9 during the study.

Participant 10

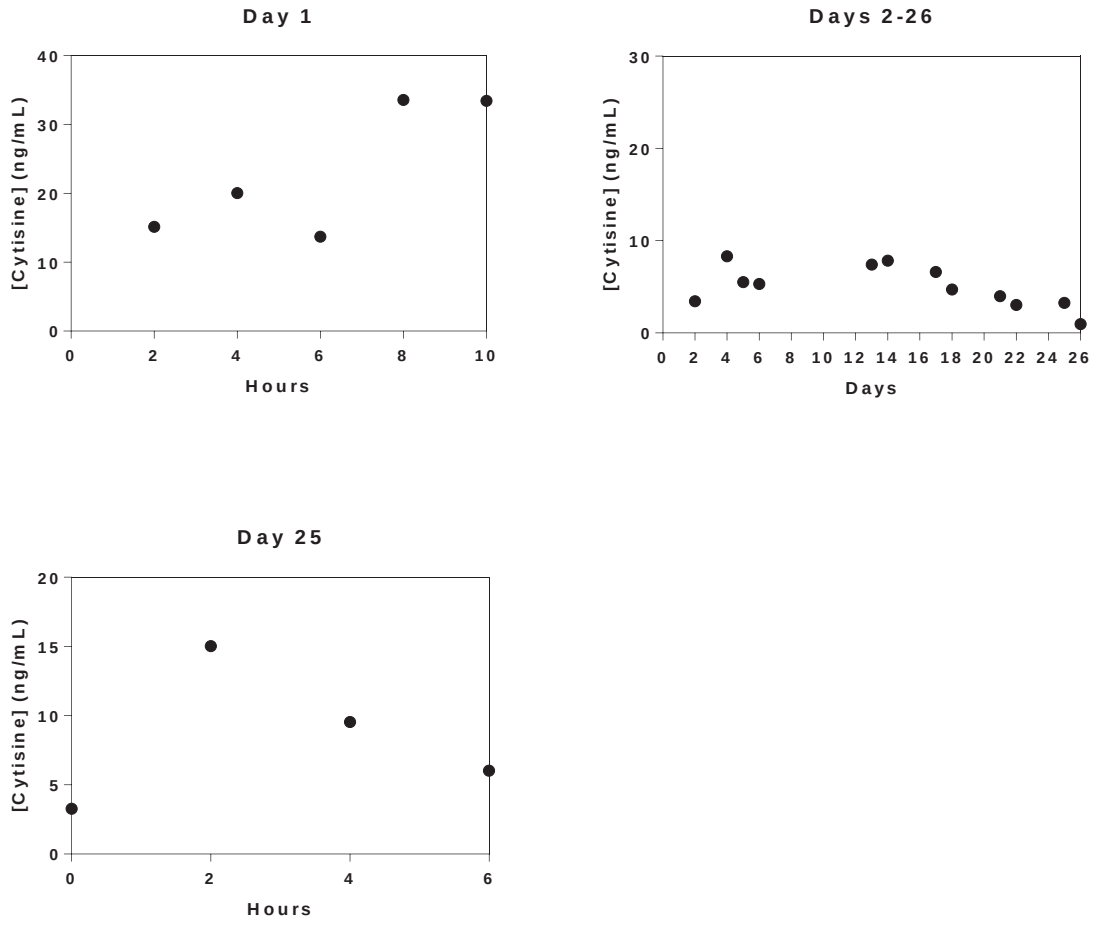

Figure 9.10: Trough plasma concentrations of cytisine measured in participant 10 during the study.

Participant who withdrew from the study

This subject withdrew from the study after day 6.

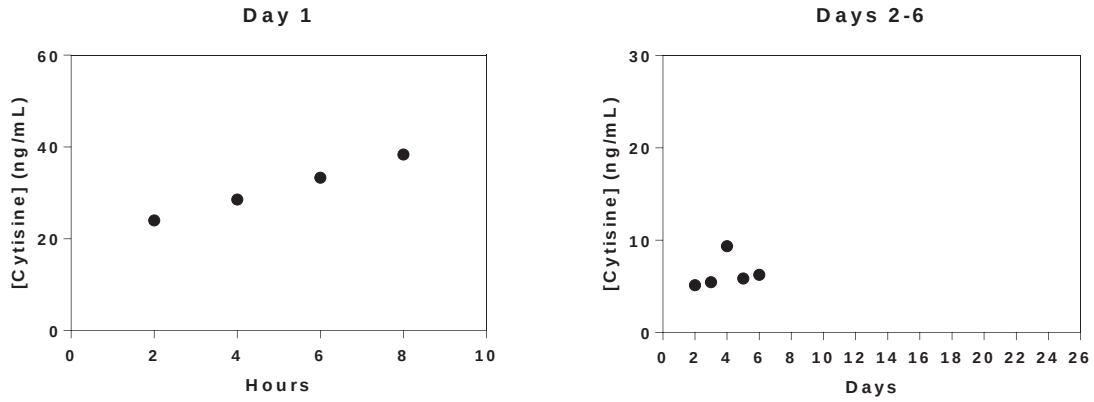

Figure 9.11: Trough plasma concentrations of cytisine measured in the participant who withdrew from the study.

Appendix 10 NONMEM code for PK model

```
$PROB cytisine
$INPUT ID DATX=DROP CLOCK=DROP TIME WT HT SEX DV AMT MDV
$DATA ..\..\data\cdraks2.csv
$EST METHOD=COND INTERACTION
MAX=9990 NSIG=3 PRINT=1
MSFO=cyt.msf
$COV

$THETA
(0,9.8,) ; POP_CL L/h
(0.1,62.8,) ; POP_V L
(0,0.5,2) FIX ; POP_TABS h
0 FIX ; POP_TLAG h
(0,0.165,1) ; RUV_CV
(0,0,) FIX ; RUV_SD mcg/L

$OMEGA
0 FIX ; PPV_CL
0 FIX ; PPV_V
0 FIX ; PPV_TABS
0 FIX ; PPV_TLAG

$SIGMA
1. FIX ; EPS1

$SUBR ADVAN2 TRANS2

$PK

GRPCL=POP_CL ;*(WT/70)**0.75
GRPV=POP_V ; *(WT/70)
GRPTABS=POP_TABS
GRPTLAG=POP_TLAG

CL=GRPCL*EXP(PPV_CL)
V=GRPV*EXP(PPV_V)
TABS=GRPTABS*EXP(PPV_TABS)
TLAG=GRPTLAG*EXP(PPV_TLAG)

;NM-TRAN
KA=LOG(2)/TABS
ALAG1=TLAG
S2=V/1000 ; mg -> mcg

$ERROR

;CONC=F
CONC=A(2)*1000/V ; mg/L -> mcg/L
```

```
PROP=CONC*RUV_CV
ADD=RUV_SD
SD=SQRT(PROP*PROP + ADD*ADD)
Y=CONC + SD*EPS1

$TABLE ID TIME AMT
CL V TABS TLAG Y
ONEHEADER NOPRINT FILE=cas.fit
```

Appendix 11 Publications and conference abstracts

Peer-reviewed journal article:

Jeong, S. H., Newcombe, D., Sheridan, J., & Tingle, M. (2014). Pharmacokinetics of cytisine, an alpha beta nicotinic receptor partial agonist, in healthy smokers following a single dose. Drug Test Anal. doi: 10.1002/dta.1707

Conference abstracts:

Jeong S., Tingle M., Sheridan J., Newcombe D. (2014). Cytisine Pharmacokinetics in Humans. Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT)- NZ Annual Meeting, Queenstown, New Zealand.

Jeong S., Tingle M., Sheridan J., Newcombe D. (2014). Cytisine - What happens to it in the body? Society for Research on Nicotine and Tobacco (SRNT) Annual Meeting Pre-conference Workshop titled Cytisine: A Globally Affordable Treatment for Tobacco Dependence? Seattle, USA.

Jeong S., Newcombe D., Sheridan J., Tingle M. (2013). Cytisine as a smoking cessation aid: measuring craving, withdrawal and mood over the recommended dosing period. Healthex, Auckland New Zealand.

Jeong S., Newcombe D., Sheridan J., Tingle M. (2013). Cytisine as a smoking cessation aid: measuring craving, withdrawal and mood over a course of treatment. Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT)- NZ Annual Meeting, Queenstown, New Zealand.

Jeong S., Sheridan J., Tingle M., Newcombe D. (2013). Cytisine – what happens to it in the human body? 4th National Addiction Research Symposium, Auckland, New Zealand.

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