Clinical Pharmacokinetics of Intranasal Scopolamine for Space Motion Sickness

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Abstract

Purpose and Specific Aims:

Space Motion Sickness (SMS) is a neurovestibular disturbance experienced by astronauts in microgravity environment, which causes acute symptoms and discomfort requiring treatment with medications during the early, mission critical time of space flight. Scopolamine (SCOP) is a historically known belladonna alkaloid used as an anticholinergic/antiemetic agent for a long time. It is a very common prescription medication for the prevention of nausea and vomiting associated with motion sickness. Earlier reports indicate that scopolamine is the most effective drug for suppressing nausea and vomiting caused by motion sickness. Bioavailability after oral administration of scopolamine is low and variable, and absorption from transdermal patch is slow and prolonged. Since the limitations of oral and other formulations of scopolamine, Pharmacotherapeutics Laboratory of Johnson Space Center developed a gel intranasal dosage form of scopolamine (INSCOP) and the bioavailability were evaluated under the Food and Drug Administration guidelines for phase I clinical trials with an Investigation New Drug (IND) application. Results showed that intranasal administration scopolamine achieves significantly higher and more reliable absolute bioavailability (83% vs 3.7%, $p < 0.05$).

The proposed research focuses on the clinical pharmacokinetics of intranasal Scopolamine used for the treatment of space motion sickness. The data used in this study have been collected in two Phase II IND clinical trials in healthy human subjects with an overall goal of developing a new formulation of scopolamine which is a rapidly acting, efficacious and safe, , countermeasure for the treatment and prevention of space motion sickness in astronauts.

The proposed specific aims are to; (1) establish PK of INSCOP with three escalating dose levels of 0.1, 0.2 and 0.4 mg; (2) estimate bioavailability of 0.2 and 0.4 mg doses of INSCOP during ambulation and simulated microgravity, Antiorthostatic Bed Rest, respectively; and (3) characterize the relationship between scopolamine concentrations in plasma, saliva and urine using co-modeling techniques.

Methods:

Aim 1: PK parameters were calculated by Winnonlin using data collected in a dose escalation pharmacokinetics (PK) study with twelve (6 male and 6 female) healthy subjects after administration of three escalating doses 0.1, 0.2, and 0.4 mgof the Investigational New Drug (IND) formulation of INSCOP administered in a completely randomized, double-blind cross-over study design.

Aim 2: A Phase II, “Randomized, Double-Blind, and Bioavailability Study of Intranasal Scopolamine in a Simulated Microgravity Environment” were established with two dose level (0.2 and 0.4 mg) in 12 normal healthy subjects (6 male/ 6 female).

Aim 3: Data from 24 subjects from the above two studies were used for PK modeling using Phoenix NLME Software. Concentrations of scopolamine in plasma, saliva and urine collected were determined using a modified LC-MS/MS method. PK parameters were derived by Phoenix WinNonlin. SAS program was used to perform statistical analysis.

Results: Aim 1: Dose-linear pharmacokinetics of scopolamine with linear increases in Cmax and AUC within the dose range (0.1mg-0.4mg). Plasma drug concentrations were significantly higher in females than in males after administration of 0.4 mg dose. Aim 2: The absorption and bioavailability of INSCOP were not changed during the microgravity

environment which means that the microgravity condition does not change the pharmacokinetics of INSCOP in human. Aim 3: The relationship among scopolamine concentrations in plasma, saliva and urine was satisfactorily described by a validated compartmental PK model and for the first time it satisfactorily predicted PK of INSCOP in plasma, saliva and urine.

Conclusion and Significance: Results of this study have a significant clinical implication in understanding the PK of intranasal scopolamine in astronauts as well as terrestrial populations receiving INSCOP for the treatment of motion sickness. Results presented here demonstrate that (1) absorption and bioavailability appear to be linear with respect to administered dose range (0.1-0.4 mg); (2) the impact of sex difference on pharmacokinetics of INSCOP was observed at high dose level of 0.4mg, indicating sex had significant influences on the clearance and volume distribution of scopolamine after IN administration; (3) Absorption and bioavailability are not significantly affected in the simulated microgravity condition; (4) PK model for scopolamine was developed which satisfactorily predicted the PK of INSCOP in plasma, saliva and urine. . Therefore, our findings support the clinical utility of INSCOP for the treatment of space motion sickness for astronauts.

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List of Abbreviations

AMB	Ambulatory Conidition
ABR	Antiorthostatic Bed Rest (Microgravity analog Condition)
AUC	Area under the Plasma Concentration-Time Curve
AUXu	Area under the Urinary Excretion Rate-Time Curve;
AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
BMI	Body Mass Index
CL/F	Apparent Total Body Clearance
C_{max}	Maximum Plasma Concentration;
CV	Coefficient of Variation
Fe	Fraction Excreted Unchanged in Urine
IM	Intramuscular
IN	Intranasal
INSCOP	Intranasal Scopolamine
I.V.	Intravenous
Ka	Absorption Rate Constant
Ka_1	Plasma Compartment, Absorption Rate Constant
Ka_2	Saliva Compartment, Absorption Rate Constant
K_m	Plasma to Saliva, the Michaelis Menten Constant
K_{21}	Saliva to Plasma Rate Constant
$K_{s/p}$	Saliva/Plasma Partition Coefficient
K_{pout}	Plasma Compartment, Elimination Rate Constant
K_{sout}	Saliva Compartment, Elimination Rate Constant

K_{el}	Plasma to Urine Rate Constant
LLOQ	Low Limit of Quantification
MW	Molecular Weight
SMS	Apace Motion Sickness
SCOP	Scopolamine
SCOP-G	Scopolamine glucuronide
SD	Standard Deviation
SE	Standard Error.
SC	Subcutaneous
SECS	Saliva Excretion Classification System
t½	Terminal Elimination Half-Llife
Tmax	Time to Reach Maximum Concentration
Vd/F	Apparent Volume of Distribution
Vmax	Plasma to Saliva, Maximum Rate
Xu	Total Amount of INSCOP Excreted in the Urine
-2LL	Minus Twice the Log of the Likelihood

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Chapter 1 Literature Review

1.1. Space motion sickness

Of all the neurovestibular disturbances experienced by astronauts immediately after exposure to the microgravity environment during space flight, Space Motion Sickness (SMS) causes acute symptoms and discomfort requiring treatment with medications during the early, mission critical time of space flight. Nearly 40% of all medications used by astronauts during flight has been for the treatment of SMS (Putcha et al., 1999).

Approximately up to 80% of Space Shuttle crewmembers experience symptoms of SMS during the first few days of flight (Reschke et al., 1998). These subjective symptoms can include pallor, malaise, anorexia, headache, fatigue, lack of motivation, impaired concentration, stomach awareness and vomiting (Reschke et al., 1994; Bagian et al., 1994). The severity of these symptoms can be debilitating and are most severe early in spaceflight beginning on the first day of microgravity and continuing for several days; later in flight, frequency of SMS and severity of symptoms appear to diminish.

The cause of motion sickness is unknown, but there are two hypotheses to be proposed: the “fluid shift” hypothesis and the “sensory conflict” hypothesis. From the fluid shift hypothesis, after entering microgravity the human body could lose of hydrostatic pressure in the lower part of the body which could induce the head ward shifting of body fluids resulting in space motion sickness. This hypothesis was largely discounted because space motion sickness symptoms were not occur in simulation models that produce head ward fluid shifts (Oman, 1998). Several studies have demonstrated that Sensory conflict is the primary cause of space motion sickness at the beginning and end

of space missions (Oman, 1987; Reschke et al., 1998; Stroud et al., 2005). Sensory conflict is thought to arise from a mismatch between expected and actual sensory inputs from the vestibular system, this mismatch causes a loss of environmental 'calibration' resulting in disorientation and motion sickness.

The underlying mechanisms responsible for inducing the symptoms of space motion sickness is not clearly elucidated. However, some studies focused on the mechanism revealed that a vestibular mediated cerebral vasoconstriction might precede nausea (Serrador et al., 2005).

Because the frequency and severity of SMS symptoms have led to restrictions on extravehicular activities (no sooner than 72 hours after launch) and mission duration (no less than 3 days), pharmacological countermeasures are mandatory and necessary. Astronauts use a variety of medications to prevent or reduce severity of SMS symptoms. Promethazine (PMZ) has been the primary pharmacologic countermeasure used to treat SMS since 1988 and, although effective, it has long-lasting side effects that include drowsiness and dry mouth. Seventy five percent (75%) of crewmembers who take PMZ during flight experience drowsiness (Bagian et al., 1994). The sedative side effect is undesirable and potentially dangerous during missions especially in an emergency, or during other mission-critical activities like Shuttle-station docking which demand optimal alertness and cognitive function. Similarly, another commonly used medication for motion sickness, Diphenhydramine, also has sedative side effects. Although it is perceived by the crew that the benefit of PMZ treatment for SMS outweighs the risk from sedative side effects of the drug, a potential for untoward adverse event still exists. Paule et al. (2004) did the examinations of the effects of drug courtermeasures for space motion sickness on working memory in humans, the results suggested that the rank order of the drugs with best cognitive profiles is Meclizine > Scopolamine >

Promethazine > Lorazepam. In our study, we focused on a new formulation of scopolamine compounded by NASA for the treatment of space motion sickness.

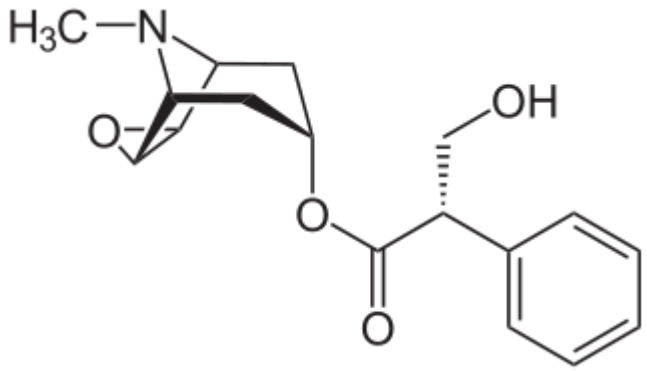
1.2. Scopolamine

In the 1880s, the German scientist Albert Ladenburg isolated scopolamine and its purified forms (such as various salts, including hydrochloride, hydrobromide, hydroiodide and sulfate) from the root of Scopolia atro-pinoides. Based on clinical investigations of scopolamine for a long time, it is used as an anticholinergic/antiemetic for the treatment of nausea, vomiting and motion sickness. Earlier reports indicate that scopolamine is the most effective drug for suppressing nausea and vomiting caused by motion sickness (American hospital formulatory service 1995 Drug information; Graybiel, 1979; Chinn et al., 1955). Wood and Graybiel (1968) compared 16 drugs and drug combinations administered orally for the prevention of motion sickness induced by slow rotating room and found the most effective treatment for motion sickness to be a combination dose of scopolamine and dextroamphetamine. Another recent study also compared the efficacy of current choices of motion sickness medications and reported that scopolamine was the most efficacious treatment for suppression of both nausea and vomit reflex induced by Vertical Rotating Chair (VRC). Of the medications tested in the study, only scopolamine had a positive treatment effect of prolongation of mean duration of rotation time in the VRC. Additionally, accuracy of Delayed Matching-to-Sample Test (DMTST), a short term memory and attention test, was found to be unaffected by scopolamine whereas other medications negatively affected DMTST scores; these results confirm that scopolamine does not impair cognitive performance like the others tested in this study (Dornhoffer et al., 2003). Based on these scientific reports and operational experience from the Navy, NASA's Reduced Gravity Office uses scopolamine tablets (0.8 mg) and

scopolamine combined with dextroamphetamine (0.8 / 10 mg) dispensed in a capsule for the treatment of motion sickness on board DC-9 parabolic flights, a microgravity analog environment for training astronauts and testing flight equipment.

1.2.1. Physicochemical properties

Scopolamine has a molecular weight of 303.4 and the structural formula is shown in **Figure 1.** Scopolamine has a weak basic character (pKa = 7.6) and a good lipid solubility expressed by a partition coefficient of 1.2 (n-octanol : buffer pH 7.4). The melt point for scopolamine is 59 ºC and the density is 1.31.



Scopolamine

Figure 1. Chemical Structure of Scopolamine

1.2.2. Pharmacology

Scopolamine is a muscarinic antagonist which has a similar structure with the neurotransmitter acetylcholine. Scopolamine competitively antagonizes the effect of acetylcholine muscarinic acetylcholine receptors by occupying postsynaptic receptor sites and is thus classified as an anticholinergic. Scopolamine can be used as an anesthetic premedication, in urinary incontinence and in motion sickness. So far the mechanism of scopolamine to prevent motion sickness is not clear. It is believe that Scopolamine prevents communication between the nerves of the vestibule and the vomiting center in the brain by blocking the action of acetylcholine. Scopolamine also may work directly on the vomiting center. Scopolamine must be taken before the onset of motion sickness to be effective.

1.2.3. Traditional formulations of scopolamine

Scopolamine is widely used as an anticholinergic agent in various forms including intraveneous (IV) to oral and transdermal form.

1.2.3.1 Oral tablet

A tablet formulation, Scopase, an over the counter medication, is no longer available. Scopolamine has been reported to have a low absolute bioavailability of approximately 4 % from an oral dose given as a tablet (Putcha et al, 1996) because of extensive first pass metabolism of the drug after oral administration. The first pass metabolism can also increase the time to reach therapeutic concentration (75-90 min). Because of the low bioavailability and lack of efficacy after symptom onset, NASA removed the oral tablet of scopolamine from astronaut's formulary.

1.2.3.2 Transdermal scopolamine

Scopolamine was the first drug to be made commercially available as a transdermal therapeutic system (TTS) delivering drug (‘‘Scopoderm’’ TTS patches, Novartis). The transdermal therapeutic system for scopolamine produced a constant rate of delivery of scopolamine through intact skin. The schematic representation of the transdermal delivery system for scopolamine placed on the skin surface was shown in **Figure 2** (Shaw et al., 1983, Chandrasekaran et al., 1983). The transdermal patch is available by prescription for use by travelers who may experience low to medium severity of motion sickness. The patch is a low dose, sustained release dosage form intended for treatment over a long period of 3 days. Transdermal scopolamine avoids first-pass metabolism, but several studies reveal that the patch has extremely slow absorption and extended time to reach therapeutic concentration (8-12 hrs) (Fung, et al., 2003; Nachum et al., 2001; Parrott, 1989). Another study also indicated that transdermal patch was notoriously inconsistent in rate and amount of drug released (Nachum et al., 2006).

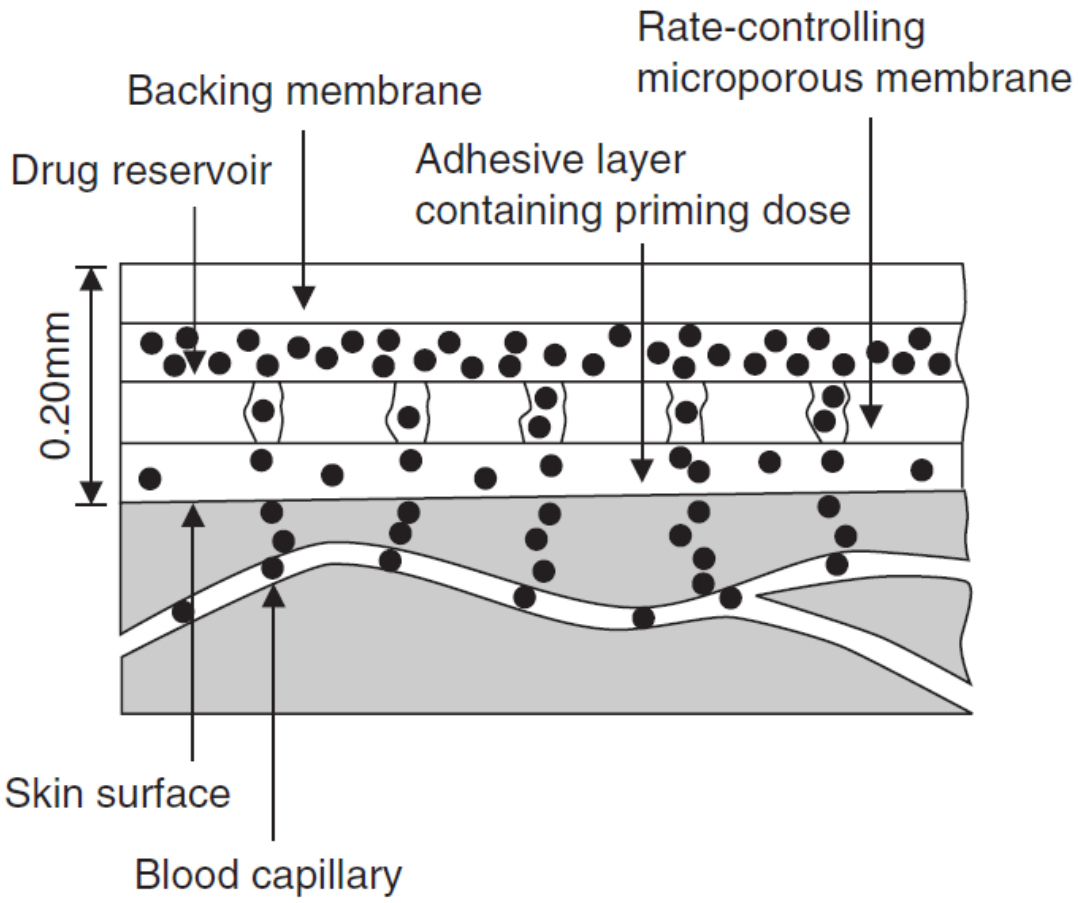


Figure 2 Schematic representationof the transdermal delivery system for scopolamine placed on the skin surface. (Shaw et al., 1983, Chandrasekaran et al., 1983)

1.2.3.3 Other administration routes of scopolamine

There are other administration route of scopolamine including intravenous and intramuscular. Studied shown that IV scopolamine has 100% bioavailability with quickly absorption and efficacy, but the IV and IM are not conducive for use in space and other remote environments (Brand, 1970; Ebert, Siepmann, Oertel,Wesnes & Kirch, 1998; Putcha et al., 1996).

1.2.4. Intranasal scopolamine

Intranasal drug delivery is a non-invasive route for drugs and is a useful and reliable alternative to oral and parenteral routes. Intranasal administration of drugs has shown promise for rapid onset of medication effect since it eliminates gut wall metabolism as well as hepatic first-pass metabolism. It also enhances the absorption, efficacy and bioavailability with fewer side effects (Chien et al. 2008).

At 1950s, Tonndorf and Chinn (Tonndorf et al., 1953; Chinn et al., 1955) started to study the nasal administration of scopolamine. The results shown that there was a rapid absorption after nasal administration with comparable efficacy after subcutaneous injection. After the modern intranasal drug formulation and medication delivery devices were vastly improved, NASA developed an aqueous intranasal dosage form of scopolamine with stability and reliability. The absolute bioavailability of intranasal scopolamine was assessed in healthy human subjects in a phase I Investigational New Drug (IND) study under FDA sponsorship. Results of this study indicated that intranasal administration of scopolamine facilitates rapid absorption with significantly higher and more reliable absolute bioavailability than an equivalent oral dose(83% vs 3.7%, $p < 0.05$) (Putcha et al. 1996). The Mean plasma concentration-time profiles after IV, IN, and PO administration of a 0.4-mg dose of scopolamine to normal subjects are presented in **Figure 3**.

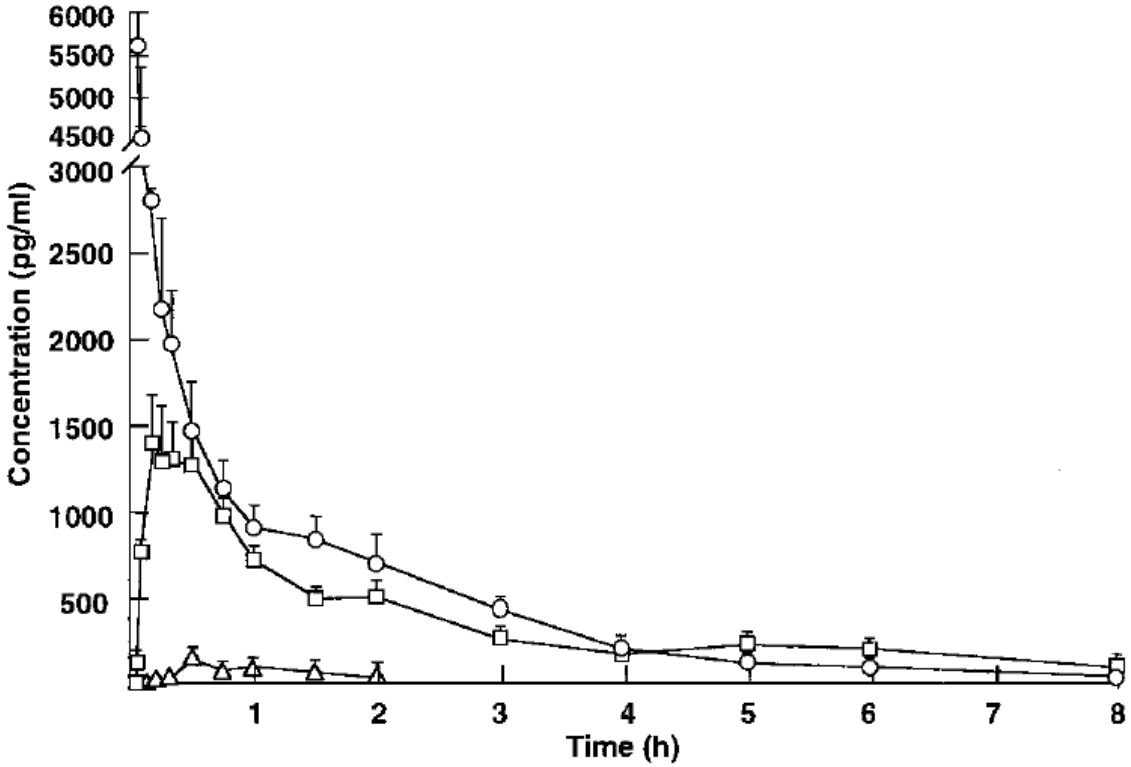


Figure 3 Scopolamine concentrations in plasma after intravenous (O), intranasal (□), or oral (Δ) administration of a 0.4-mg dose to healthy subjects. (Putcha et al. 1996)

1.2.5. Pharmacokinetics and pharmacodynamics of clinical use of scopolamine

1.2.5.1 Pharmacokinetics

The PK parameters of scopolamine depend on dosage forms. The pharmacokinetic parameters of scopolamine after IV infusion, oral, subcutaneous and intramuscular administration were shown in **Table 1** (Ulf D. Renner, Reinhard Oertel, and Wilhelm Kirch, 2005). The plasma protein binding of scopolamine was studied in rats with the low value of 30 ±10% (Nakashima et al. 1993). Little data concerning its metabolism and renal excretion in healthy volunteers and patients have been published up to now. Although the metabolic and excretory fate of scopolamine has not been fully determined, the drug is thought to be almost completely metabolized (principally by conjugation) in the liver and excreted in urine. Scopolamine has a complex metabolism pathway base on the research of metabolites excreted in urine of mammals or man (**Figure 4**). The excretion of scopolamine in human urine depend on dosage form. After oral administration, only 2.6% of the drug is excreted in urine in a pharmacologically active form (Kanto J, Kentala E, Kaila T, et al.1989). After IV infusion, a recovery of 3% of scopolamine in human urine was found and after an incubation with b-glucuronidase/sulfatase, the amount of scopolamine in human urine increased from 3% to 30% (Ebert U, Oertel R, Kirch W.2000; Kentala E, Kaila T, Ali-Melkkila T, et al., 1990). With a transdermal administration, the recovery of 10% was found in human urine.

Table 1. The Most Important Pharmacokinetic Data of Scopolamine after multiple administration route(Ulf D. Renner, Reinhard Oertel, and Wilhelm Kirch, 2005)

TABLE 1. The Most Important Pharmaco Kinetic Data

Application	IV	Oral	SC	IM
Dose (mg)	0.5	0.5	0.4	0.5
C_{max} (ng/mL)	5.00 ± 0.43	0.54 ± 0.10	3.27	0.96 ± 0.17
t_{max} (min)	5.0	23.5 ± 8.2	14.6	18.5 ± 4.7
AUC (ng∗min/mL)	369.4 ± 2.2	50.8 ± 1.76	158.2	81.3 ± 11.2
$t_{1/2}$ (min)	68.7 ± 1.0	63.7 ± 1.3	213	69.1 ± 8.0
F (%)	100	13 ± 1	ND	ND

ND, no data.

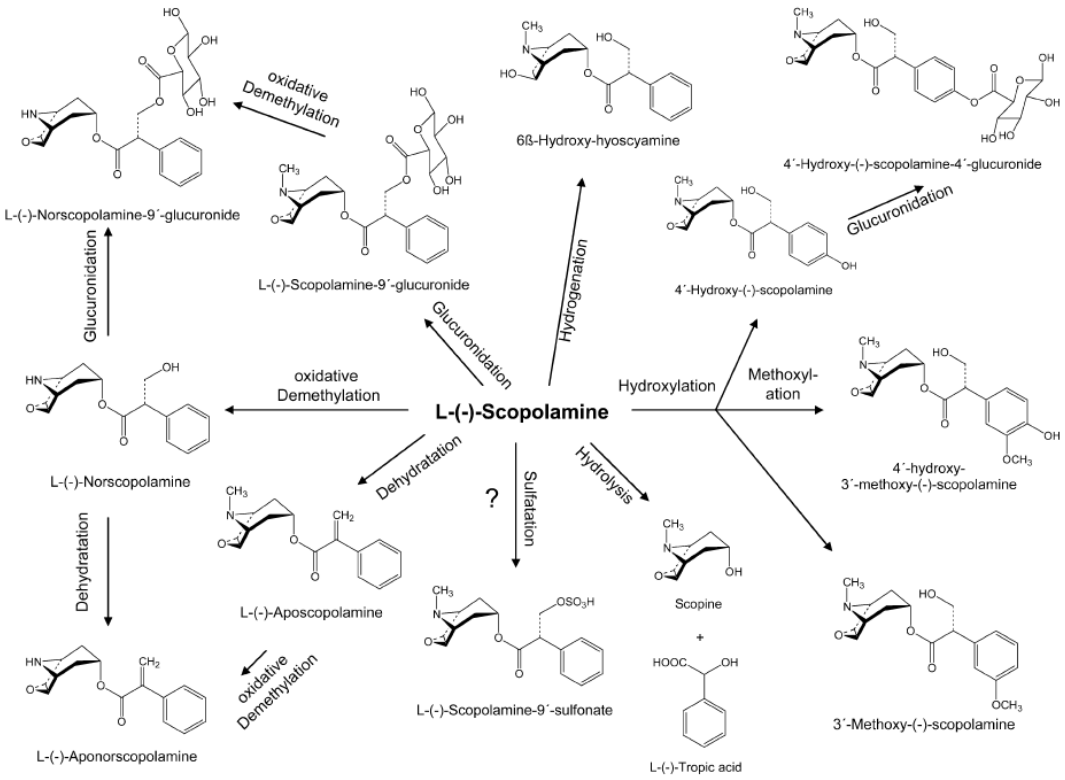


Figure 4. Metabolic Pathways of Scopolamine(Ulf D. Renner et al., 2005)

1.2.5.2 Pharmacodynamics

After oral administration of scopolamine, the effectiveness in preventing motion sickness shown within 0.5 hours and last for 6 hours. The transdermal administration of the same drug is effective over a period of 72 hours, but the effect is not achieved until 6 to 8 hours post dose (Nachum Z, Shahal B, Shupak A, et al.2001). After IV infusion, scopolamine significantly decreased subjective alertness in both male and female subjects (Ebert U, Oertel R, Kirch W, 2000).

1.2.5.3 Adverse Effects

The common adverse effects of scopolamine include fatigue, dry mouth, dreamless sleep (reduction in rapid eye movement, REM), amnesia, vertigo, vestibular depression. The adverse effects are dose dependent and associated with administration routes. The effects of scopolamine on the cardiovascular system are complex and dose dependent. Low doses of the alkaloid give rise to a slowing of heart rate, whereas higher doses produce an increase in heart rate dose-dependently (Clissold SP, Heel RC, 1985). Scopolamine can rise the inhibition of gastric salivation and secretion and can cause dryness in the respiratory tract which can cause the dry mouth (Mirakhur RK, Dundee JW. Gycopyrrolate, 1983), additionally scopolamine could decrease the motility of stomach and esophagus. Scopolamine produces mydriasis and cycloplegia, studies shown that eye functions may be impaired up to 7–12 days when scopolamine is administered locally (Clissold SP, Heel RC, 1985). After IV infusion of scopolamine, the sweat gland activity is reduced (Turner P.1980).

1.3. Physiologic and pharmacokinetic changes in space

The exposure to the microgravity environment induces several physiologic changes in human body. Some of these changes, including in cardiovascular, gastrointestinal, muscle, hepatic metabolism, body fluid and renal systems, may influence the pharmacokinetics (absorption, distribution, metabolism and elimination) of drugs administrated in space. These changes in pharmacokinetics of drugs may cause reduced pharmacological activity or increase of adverse effects. The overall physiological changes are summarized in **Figure 5** (Nicogossian et al).

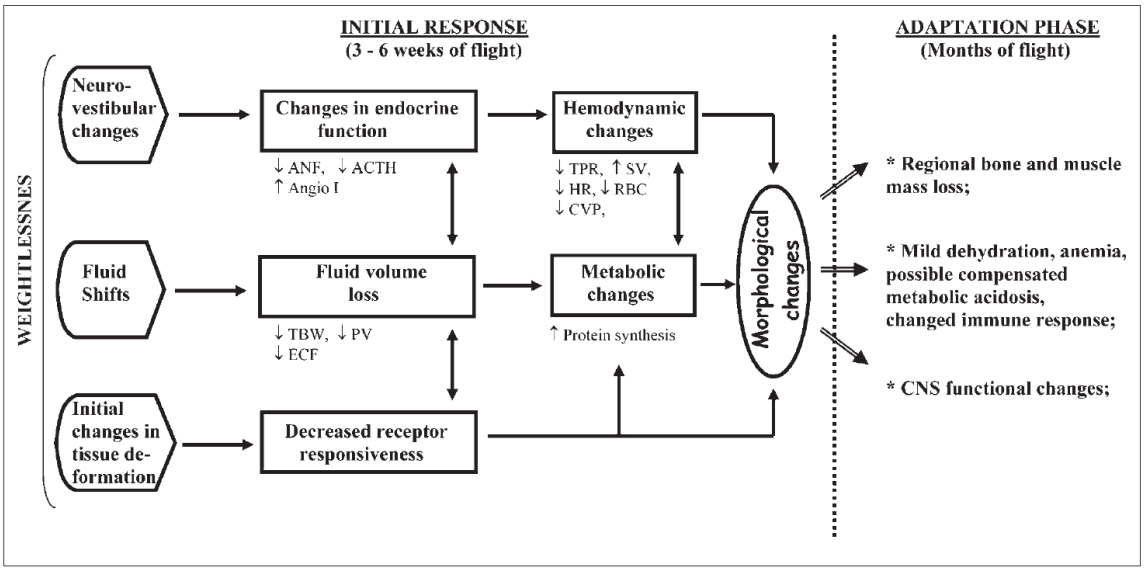


Figure 5. Schematic representation of human adaptation to weightlessness. (Nicogossian et al., 1994).

1.3.1. Changes in cardiovascular systems

During the exposure in microgravity environment, cardiovascular adaptation is present which the well-studied responds of body to weightlessness is. The mechanism of cardiovascular adaptation involves body fluid balance, vascular adaptation and renal hemodynamics.

The reports about Arterial blood pressure (BP) are not always in consistency during spaceflight, hypotension commonly occurs during orthostatic stress after spaceflight. Vestibular adaptive changes present after the return to earth from space which influence the sympathetic nervous system and cardiovascular control. A recently study in astronauts by Gundel et al (2002) indicated that a 20% decrease in heart rates after 438 days stayed in space. While it was reported in early 1990s by Charles JB (1994) that heart rate and mean blood pressure (both systolic and diastolic) were elevated and remained higher than preflight levels during the mission.

1.3.2. Changes in body fluid and renal systems

Limited data was reported on body fluid and renal hemodynamics in real space flights, a lot of data were generated from ground-base models or compared post flight data with preflight data without inflight observation. Even there are inconsistent report about sodium and electrolyte balance in microgravity, there is an agreement that the blood volume and extracellular fluid decrease and overall fluid deficit during space flight.

There are several hypothesis to explain the reduction of plasma volumes, the new hypothesis conducted by Norsk et al (2001) is presented in the **Figure 6.**

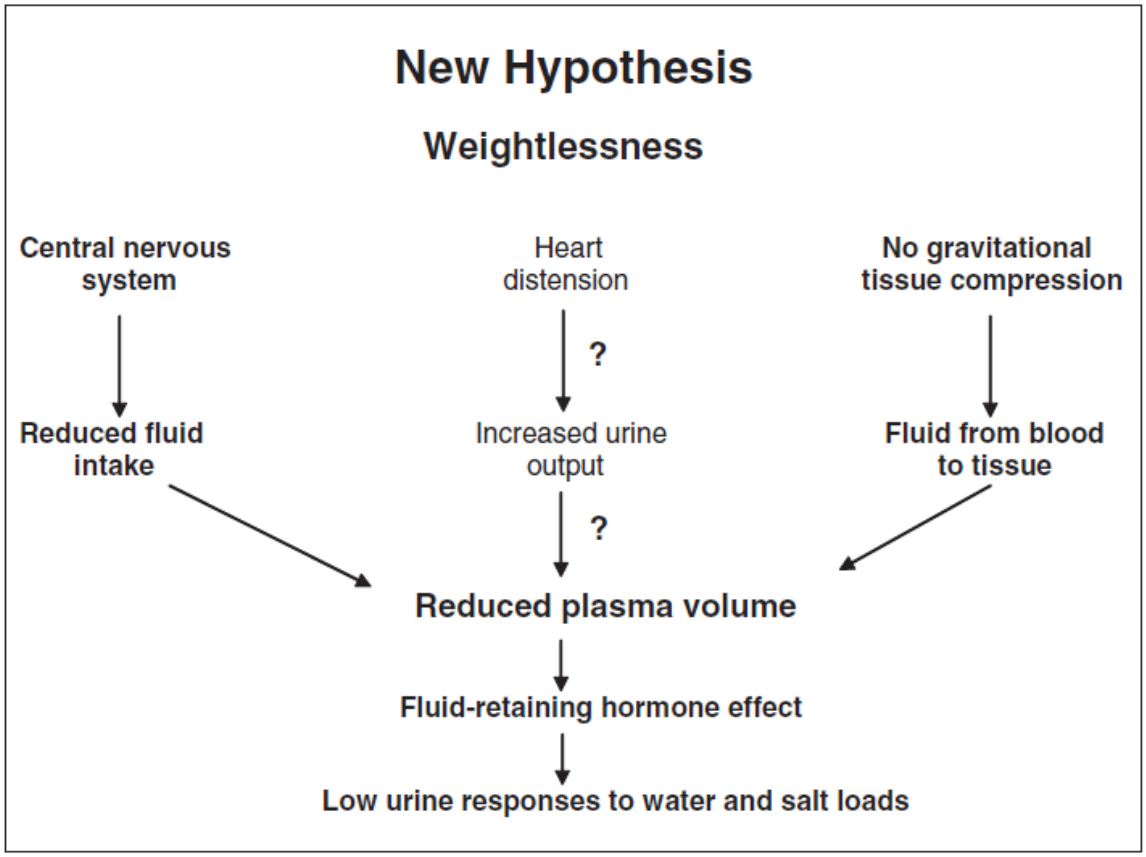


Figure 6. New hypothesis on how blood volume is decreased during space flight (Norsk et al., 2001)

1.3.3. Changes in muscle and bone

Both decreased muscle strength and bone decalcification were observed during space flight, both in humans and animals. Leonard JI (1983) reported that Muscle atrophy and loss in lean body mass, associated with a decline in peak force and power. Later in 2001, Fitts RH reported that a 37% decline in muscle mass was observed in rats after 1 week of microgravity.

The loss of bone mass, especially in weight-bearing bones were observed in space flight. A site-specific loss of bone mineral density was as much as 1% to 2% per month for astronauts in microgravity environment. Two studies base on an anti-orthostatic bed rest(ABR) have suggested that bone formation may be decreased partially as a result of reduced osteoblast function, whereas bone reabsorption is transitorily increased or unaltered (Loomer PM, 2001; Carmeliet G, 2001).

1.3.4. Changes in enzymes and metabolism

A variety of drug metabolizing enzymes were studied in animals models which are subjected to space flights. Several studies shown the transient increase of intestinal digestive enzymes during space flights in rat model (Groza P, 1983; 1987). Another study focused on the rats flown on cosmos, shown that the amount of microsomal P-450 and the activity of aniline hydroxylase and ethylmorphine-N-demethylase, cytochrome P-450-dependent enzymes, were decreased (Merrill AH Jr, 1990). In rats suspended model study, the levels of P-glycoprotein, CYPC211 and CYP2E1 were found significantly reduced, but liver and intestinal CYP3A2 was not affected (Lu SK, 2002).

1.3.5. Other physiological changes

There are other physiological changes present during the space flight, which includes changes in organ perfusion, immunologic function, red blood cell mass, hormone levels and neurovestibular performance.

1.3.6. Physiologically related PK changes

The physiological changes in humans in may influence the pharmacokinetics (absorption, distribution, metabolism and elimination) of drugs administrated in space. Knowledge of the physiological and pharmacokinetic aspects under microgravity would be useful in making appropriate dosing recommendations.

1.3.6.1 Absorption

There are many factors that can influence the drug absorption especially by oral administration. The factors include gastric emptying rate, intestinal transit time, hepatic first-pass metabolism, and gastrointestinal and hepatic blood flow. Gastric emptying has been shown to be inhibited during motion sickness. Intestinal transit rate may increase alone the small intestine by decreasing the dimensionless ratio of gravitational forces to viscous forces. Blood flow to the liver is a major determinant of the metabolism of drugs with a high extraction ratio, a decrease in blood flow to the liver reduces the metabolism of such drugs with a high extraction ratio in space.

1.3.6.2 Distribution

The decrease of total body fluid and plasma volume could result in a decrease in the volume of distribution, and the muscle loss in space may also causes the alternation of the volume of distribution of drug administrated in space.

1.3.6.3 Metabolism and elimination

Since blood flow were reduced in in space, the reduction of blood flow to the organs of elimination (liver and kidney) may result in slower metabolism or excretion, which may lead to greater and longer-lasting drug activity. The activities of variety enzyme were observed in space which could cause the alternation of metabolism of drug in space.

1.4. Pharmacokinetic study in Saliva

Saliva sampling is now increasingly applied in clinical practice. When compared with blood sampling, it is easy for accessory and collection. Salivary excretion of some drugs has been reported previously as a good indicator for drug bioavailability, therapeutic drug monitoring and pharmacokinetics. In this section ,we discuss the physiology of saliva secretion and drug transfer to saliva.

1.4.1. Salivary glands

The salivary glands are composed of acini, in which the initial or primary saliva is produced. There are three pairs of major glands which are presented in **Figure 7**(A. Van Nieuw Amerongen, 2004). Every type of salivary gland produces a typical secrete. The glandula parotis produces a serous fluid, the glandula Submandibularis produces a sero-mucous secrete, while the glandula Sublingualis secretes only mucous saliva (E.C.I. Veerman1996; J.H. Poulsen 1998). Saliva glands can produce the largest portion of saliva with the formation of enzymes, mucin, and other components of saliva. Another function of saliva glands is the exchange of inorganic ions with blood.

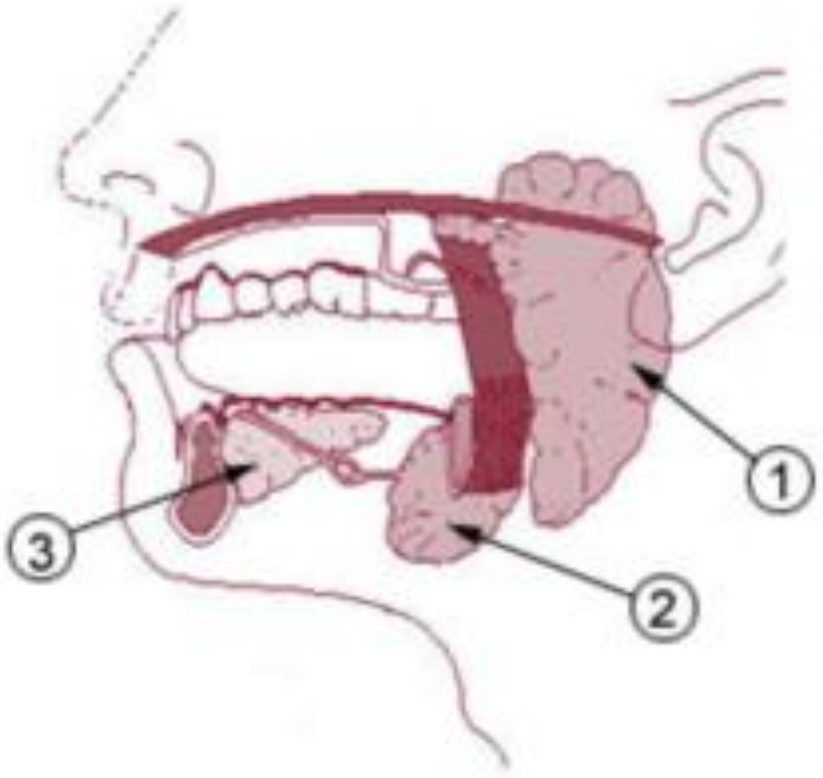


Figure 7 The Anatomical Positioning of The Three Pairs of Large Salivary Glands; The Glandula Parotis (1), the glandula submandibularis (2) and the glandula sublingualis (3)(A. Van Nieuw Amerongen, 2004)

1.4.2. Mechanisms of drug transfer to saliva

As we know if a drug circulating in plasma transport into the saliva, it must pass through the capillary wall, the basement membrane and the membrane of the glandular epithelial cells. The rate-determining step for this transportation is the passage of the drug through the lipophilic layer of the epithelial membrane. For such a passage to occur, drug must show a degree of lipophilicity. However, saliva is not a simple ultrafiltrate of plasma, it is a complex fluid formed by different mechanisms: by a passive diffusion process, by ultrafiltration through pores in the membrane, by an active process against a concentration gradient. All three mechanism of drug transfer from blood to saliva were presented in **Figure 8.**

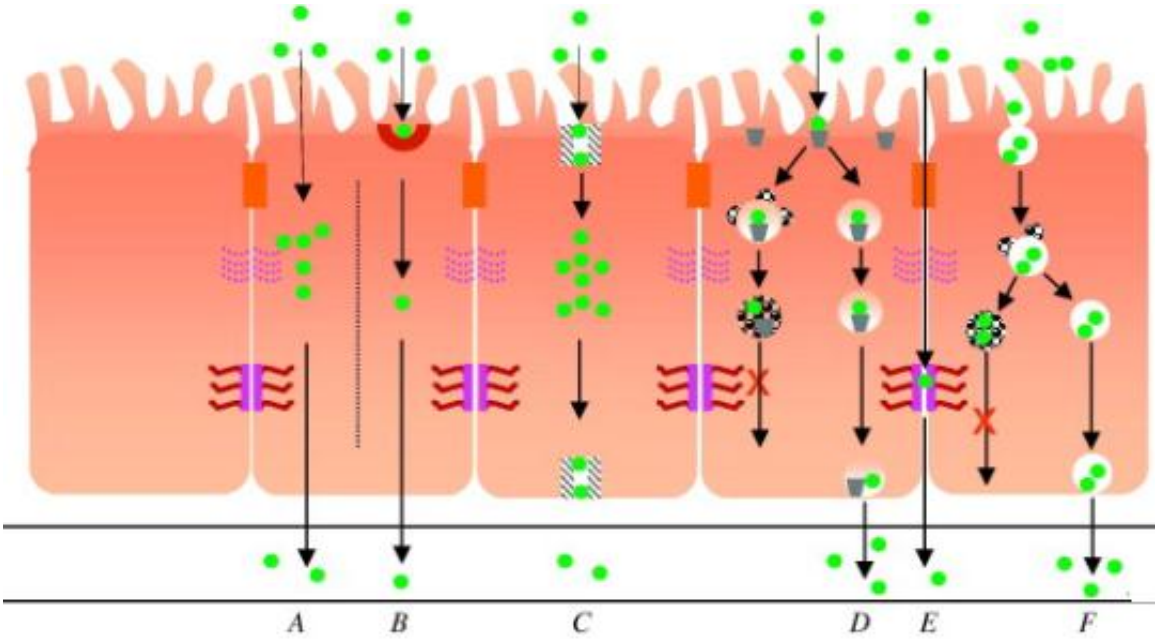


Figure 8 Summary of mechanism of drug transfer from blood to saliva. A: Passive diffusion; B: Active transport and E: Paracellular transport. (A. Van Nieuw Amerongen, 2004)

1.4.2.1 Passive diffusion

Passive diffusion appears to be the main way of drugs enter to saliva, this process is refer to the transfer of drug molecules down a concentration gradient between epithelial membranes without energy. It has been shown that most drug, especially highly lipid-soluble drugs, enter the saliva via this way.

1.4.2.2 Ultrafiltration (or paracellular transport)

Paracellular transport is characterized by the transfer of drugs across an epithelium by passing through the intercellular space between the cells. Small polar molecules is preferred with the mechanism while this mechanism is restricted to compounds with a molecular weight (MW) of less than about 300 Da.

1.4.2.3 Active transport

Unlike passive diffusion and paracellular transport, active transport is refer to the movement of the drug across the cell membranes in the direction against their concentration gradient. It involves the use of energy and can become saturated.

1.4.3. Salivary Excretion Classification System

A study conducted by Nasir Idkaidek and Tawfiq Arafat (2012) suggested a salivary excretion classification system for drug with different permeability and fraction unbound to plasma proteins (fu). Thirteen drugs with different pharmacokinetic, physicochemical and pharmacodynamics properties were selected randomly from ongoing bioequivalence studies in humans, and drug protein binding and membrane permeability were investigated. Salivary Excretion Classification System (SECS) are presented in **Table 2**. According to the mechanism of drug transfer to saliva and SECS, we summarize the eight drugs in **Table 3** for the classification.

Table 2. Salivary Excretion Classification System (SECS) According to Drug Permeability (Peff) and Fraction Unbound to Plasma Proteins (fu). (Nasir Idkaidek and Tawfiq Arafat, 2012)

	P_{eff}	fu	salivary excretion
class I	high	high	yes
class II	low	high	yes
class III	high	low	yes
class IV	low	low	no

Table 3. Summary of Eight Drugs of Saliva Excretion Classification System (SECS) and Transfer Mechanism.

Drug Name	**Mechanisms**	**MW**	**Lipid Solubility**
Cinacalcet	Passive Diffusion	393	High
Sitagliptin	Active Transport	523	Low
Tolterodine	Active Transport	475.6	Low
HCT	Passive Diffusion	297	High
Metformin	Paracellular Transport	165	Low
Cloxacillin	Active Transport	457.6	Limited
Azithromycin	Passive Diffusion	748.9	High
Rosuvastatin	Active or Passive	481.5	Low

Summary

This survey of the literature reveals that Scopolamine has been suggested to be suitable for the treatment and prevention of space motion sickness in astronauts. However, the traditional dosage forms of scopolamine have disadvantages including low absolute bioavailability and inconsistent drug release rate that make the traditional formulations of scopolamine not suitable for the using in space for astronauts. The physiological changes in astronauts when exposed to the microgravity environment, could induce alterations in both pharmacokinetics and pharmacodynamics of scopolamine in space. Development of intranasal dosing may offer an efficacious alternative for the treatment of space motion sickness. Additionally, effect of microgravity on pharmacokinetics of intranasally administered scopolamine also should be characterized. In order to assess the PK or dosing of scopolamine in space, the development of PK model for INSCOP in plasma, saliva and urine will offer noninvasive alternatives for such assessments. With the development of a predictive PK model, plasma concentrations of SCOP can be predicted using saliva samples which is safe and more convenient for astronauts to collect than blood sampling in space.

Chapter 2 Objective and Specific Aims

2.1. Hypotheses

Our long-term goal is to have a new formulation of scopolamine which have a safe, rapidly acting, efficacious countermeasure for the treatment and prevention of space motion sickness in astronauts. We hypothesize that the intranasal formulation of scopolamine could offer such a countermeasure for the treatment and prevention of space motion sickness in astronauts.

2.2. Objective

Our proposed research focused on analysis and PK model development using data collected during clinical studies with intranasal scopolamine in healthy human subjects. The overall objective of the study is to develop an intranasal gel formulation of scopolamine which have a safe, rapidly action, efficacious countermeasure for the treatment and prevention of space motion sickness in astronauts. In our study, we conducted phase II clinical trials for INSCOP to study the drug exposure and safety. The rationale is that the traditional dosage forms of scopolamine have disadvantages, the transdermal patch is inconsistent in rate and amount of drug release, while tablet dosage form has a low absolute bioavailability. Since these disadvantages of the formulations for the treatment of SMS, we started our project to formulate an intranasal gel scopolamine.

2.3. Specific Aims

Toward our goal, three specific aims were proposed: (1) To establish PK of INSCOP with three escalating dose levels of 0.1, 0.2 and 0.4 mg; (2) To estimate the bioavailability of 0.2 and 0.4 mg doses of INSCOP during ambulation and simulated microgravity, antiorthostatic bed rest, respectively; and (3) To characterize the relationship among scopolamine concentrations in plasma, saliva and urine by performing co-modeling**.**

2.3.1. To establish PK of INSCOP with three escalating dose levels of 0.1, 0.2 and 0.4 mg

- To establish plasma concentration-time profiles of INSCOP and scopolamine glucuronide conjugate in healthy human subjects at the three dose levels, according to the approved clinical protocol by the NASA Johnson Space Center Committee for the Protection of Human Subjects and the Investigational Review Board of MDS Pharma Services (Lincoln, Nebraska).
- To determine the pharmacokinetic parameters using 1-compartment PK model analysis by WinNonlin.
- To establish the dose linearity of INSCOP indicated by C_{max} and AUC by statistical analysis.
- To evaluate the sex impact on PK of INSCOP at 0.4 mg dose by population PK analysis.

2.3.2. To estimate the relative bioavailability of 0.2 and 0.4 mg doses of INSCOP during ambulation and simulated microgravity, antiorthostatic bed rest, respectively

- To establish plasma concentration-time profiles of INSCOP in healthy human subjects at the two dose levels.
- To determine the pharmacokinetic parameters using 1-compartment PK model analysis by WinNonlin.
- To estimate the impact of microgravity condition on INSCOP PK by comparing the relative bioavailability and the PK parameters between the two gravity conditions using SAS programs.

2.3.3. To characterize the relationship among scopolamine concentrations in plasma, saliva and urine by performing co-modeling

- To establish saliva concentration-time profiles of INSCOP in healthy human subjects at all three dose levels
- To establish cumulative urinary excreted amount-time profiles of INSCOP in healthy human subjects at the dose levels
- To develop a compartmental PK model that can best describe the concentration of INSCOP in plasma, saliva and urine.
- To validate the best fit compartmental PK model by internal and external validations.
- To develop a compartmental PK model that can best describe the concentrations of INSCOP, as well as scopolamine glucuronide (SCOP-G) metabolite in plasma, saliva and urine.

Chapter 3 Clinical protocol and Methods

3.1. Clinical protocol

3.1.1. Subjects

- Twenty four healthy, non-smoking human subjects between 21 and 47 years of age with matching astronaut's age group, participated in the study after giving a written informed consent briefing.
- Subjects were brought into the MDS Pharma Services (Lincoln, Nebraska) Research Clinic on the night before for an overnight stay for an 8-10 h fasting and were given the first dose at approximately 8 AM the next morning.
- Subjects who had a history of nasal surgery were excluded from the study.
- Caffeinated beverages and grapefruit containing products were restricted during the study period.
- Fluid intake was monitored on study days to maintain an adequate hydration.
- Different 12 subjects were enrolled in studies for Specific Aims 1 and 2.

3.1.2. Treatments

3.1.2.1 Treatments in Specific Aim 1

- Two intranasal gel formulations with 0.1 mg and 0.2 mg of scopolamine hydrobromide, respectively, in 0.1 g of gel were custom-manufactured by Nastech Pharmaceuticals, Inc., Bothell, Washington and were dispensed in actuator pumps (Pfifer Inc).
- The carrier gel was custom compounded as a proprietary, undisclosed formulation by Nastech.
- A fully randomized double blind crossover study design was used for drug and placebo treatments with a seven-day washout period between treatments.
- Each subject received three doses of scopolamine, 0.1, 0.2 and 0.4 mg, and a placebo; the order of treatments was randomized amongst subjects.
- Treatments were administered following an 8-10 hour overnight fast starting at bed time on the day before each treatment.
- All treatments were administered by a study nurse to deliver one squirt of the gel into each nostril; for 0.1 mg dose, subjects received a squirt of placebo in one of the nostrils.

3.1.2.2 Treatments in Specific Aim 2

- Two intranasal gel formulations with 0.1 mg and 0.2 mg of scopolamine hydrobromide, respectively, in 0.1 g of gel were custom manufactured by Nastech Pharmaceuticals, Inc., Bothell, Washington and were dispensed in actuator pumps (Pfifer Inc).
- The same carrier gel Nastech was used
- Each subject received two doses of scopolamine, 0.2 and 0.4 mg and a placebo

in simulated microgravity and in normal gravity, respectively.

- A fully randomized, double blind crossover study design was used for drug and placebo treatments with a seven day washout period between treatments.

3.1.3. Sample preparation

- Serial blood samples (7 ml each) were collected, at 0, 0.083, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after each treatment, into heparinized vacutainers from an indwelling catheter (Intracath®, Becton and Dickinson) placed in the antecubital vein in the arm.
- Samples were gently mixed by inversion and centrifuged at 3,000 rpm for 15 mines to separate plasma, which was transferred into cryotubes and stored frozen at -40^{o}C until analysis.
- Serial saliva samples were collected at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after each treatment.
- Urine voids were collected at scheduled time intervals between 5 min to 24 h after dosing. The volume of the sample were recorded at the end of each collection interval.
- Samples were stored frozen at -80ºC until analyzed.

3.2. Methods

3.2.1. Equipment, Apparatuses and Software

- Waters® LC-MS/MS system (Waters Corporation, Milford, MA, USA) and Empower software was used to analyze the parent scopolamine and scopolamine glucuronide conjugate.
- Column: an Agilent Zorbax SB-CN column (50x2.1 mm, 5µm) was used for LC-MS/MS analysis.
- Phoenix WinNonlin version 1.3 (Pharsight Corp., Mountainview, CA, USA) was used for non-compartmental and compartmental pharmacokinetic data analysis. The Phoenix NLME (non-linear mixed effect) package is used on development of pharmacokinetic compartmental model and derivation of the pharmacokinetic parameters.
- Graphpad Prism version 5.02 (GraphPad Software, San Diego, CA) was used for Student's t test and one-way ANOVA test.

3.2.2. LC-MS/MS assay for quantifications of INSCOP and SCOP-G in healthy human plasma, saliva and urine samples

3.2.2.1 LC-MS/MS condition

- Separation and quantitation of scopolamine concentrations in the sample extracts was achieved using a Waters Acquity UPLC system combined with Micromass Quattro Micro^{TM} API MS/MS detector using an electrospray interface.
- Chromatographic separation was accomplished with the Agilent Zorbax SB-CN column.
- A mobile phase of methanol: 2 mM ammonium acetate 90:10 (v/v), pH adjusted to 5.0 ± 0.1, with a flow rate of 0.2 mL/min, with sample injection volume of 10 µL, and run time of 4 minutes.
- Positive ions were monitored in the Multiple Reaction Monitoring (MRM) mode for the determination of scopolamine m/z = 304.2 → 138.1 and internal standard hyoscyamine, m/z = 290.2 → 124.1.

3.2.2.2 LC-MS/MS method validation

- Precision and accuracy of the assay were acceptable with r^2=0.99 or better for the linearity established by the regression of response areas across the detected concentrations range between 100 and 1000 pg/mL.
- The LLOQ was determined as the concentration producing a peak with a signal-to-noise ratio of 10:1. The noise level was the peak area resulting from the blank sample. The LLOQ was 50 pg/mL.
- Inter and intra-day coefficients of variation were determined by QC samples containing low, medium and high INSCOP concentrations (100, 300, 750 pg/ml).
- The extraction recoveries were determined by three level concentrations of INSCOP (100, 500, 1000 pg/ml) prepared in blank human samples and reconstitution solution.

3.2.2.3 Quantification of Scopolamine and scopolamine glucuronide conjugate

- Total scopolamine concentrations, consisting of unconjugated parent scopolamine and β- glucuronide conjugate, were determined after incubation of samples with β-glucuronidase for 12 h to cleave scopolamine and glucuronide before solid phase extraction.
- Scopolamine glucuronide levels in plasma, saliva and urine were determined by difference between total and unconjugated scopolamine concentration.

3.2.3. Dose escalating pharmacokinetics studies in healthy human subjects.

3.2.3.1 Clinical study protocol

The clinical protocol was reviewed and approved by the NASA Johnson Space Center Committee as described in Section 3.1.

3.2.3.2 LC-MS/MS assay of plasma, saliva and urine samples from pharmacokinetic studies

Concentration of SCOP and SCOP-G in plasma, saliva and urine were measured by a validated LC-MS/MS method described in section 3.2.2.

3.2.3.3 One-compartmental pharmacokinetic analysis

Pharmacokinetic analysis of data was performed using one-compartmental methods with Phoenix WinNonlin version 1.3 (Pharsight Corp., Mountainview, CA, USA). The maximum plasma concentrations (C_{max}) of Scopolamine and SCOP-G, and the time to reach C_{max} (T_{max}) were derived from the individual plasma concentration profiles. The area under the curve (AUC), clearance (CL), elimination rate constant (k_e), absorption rate constant (k_a), volume of distribution (V_d) and elimination half-life ($t_{1/2}$) were determined by compartment PK analysis.

3.2.3.1 Non-compartmental PK analysis of saliva samples

Pharmacokinetic and statistical data analyses were performed using Phoenix WinNonlin version 1.3 (Pharsight Corp., Mountainview, CA, USA). WinNonlin was used to model saliva concentration versus time profiles of scopolamine (and its metabolite) using a non-compartmental model for saliva samples to estimate PK parameters. The C_{max} and T_{max} of saliva parent (INSCOP) and SCOP-G (scopolamine glururonide) were derived from the

individual saliva concentration profiles. The area under the curve (AUC), the terminal elimination half-life ($t_{1/2}$), the systemic clearance (CL) and the volume of distribution (V) were determined from saliva data using non-compartment analysis.

3.2.3.2 Non-compartmental PK analysis of urine samples

Pharmacokinetic and statistical data analyses were performed using Phoenix WinNonlin version 1.3 (Pharsight Corp., Mountainview, CA, USA). WinNonlin was used to model urine excretion rate versus time profiles of scopolamine and its SCOP-G using a non-compartmental model for urine samples to estimate the total amount excreted in the urine (Xu), the area under the urinary excretion rate-time curve (AUXu), elimination rate constant (k_e), percent of dose excreted in the urine (PDEx), and elimination half-life ($t_{1/2}$) for the three tested doses.

3.2.3.3 Statistical analysis

Scopolamine and SCOP-G concentration data and PK parameters were reported as mean and standard deviation (Mean ± SD). Dose linearity of area moment pharmacokinetic parameters was tested using SAS proc glm (Cary, NC, USA). Differences of PK parameters between sexes were determined using a non-parametric Wilcoxon-Mann-Whitney test, with the level of significance set at $\alpha=0.05$.

3.2.4. Sex impact study of INSCOP in plasma at dose of 0.4 mg

3.2.4.1 Population PK analysis

Sex impacts on INSCOP PK parameters were determined by a population covariates PK analysis study. Plasma scopolamine concentrations as a function time data collected from 12 normal healthy human subjects (6 male/6 female) were used for analysis using Phoenix NLME (nonlinear mixed-effects, version 1.3). One-compartment pharmacokinetic models with first-order elimination was selected to describe the base model. The model of ombined additive and proportional residual errors was selected and the equation is list as blow.

$$C_{observed,ij} = C_{pred,ij} \times \left(1 + \varepsilon_{p,ij}\right) + \varepsilon_{a,ij}$$

Where $C_{observed,ij}$ represents the observed concentration for individual *i* and observation *j*, $C_{pred,ij}$ represents the individual predicted concentration, and $\varepsilon_{p,ij}$ and $\varepsilon_{a,ij}$ represent the proportional and additive errors distributed following N (0, σ^2).

Sex, body weight, age were employed as covariates in the model selection. Covariates were screened for their possible influence on INSCOP PK parameters by the forward and backward stepwise modeling building approaches. Model selection and identification of variability were based on evaluation of the -2 × log likelihood (-2LL) of the data, plausible parameter estimates, adequate parameter precision, and inspection of goodness-of-fit plots. A difference in -2LL of at least 3.84 (corresponding to $p < 0.05$) was used to discriminate between competing models.

3.2.4.2 Reliability of model

The reliability of the analysis results was checked by careful examinations of diagnostic plots, including predicted versus observed concentrations and weighted residuals versus time plots.

3.2.5. Relative bioavailability study in healthy human subjects under ambulatory and simulated microgravity conditions

3.2.5.1 Clinical study protocol

The clinical protocol was reviewed and approved by the NASA Johnson Space Center Committee as described in Section 3.1.

3.2.5.2 LC-MS/MS assay of plasma and urine samples from pharmacokinetic studies

Concentrations of Scopolamine in plasma and saliva were measured by the validated LC-MS/MS method described in Section 3.2.2.

3.2.5.3 One-compartmental pharmacokinetic analysis

Pharmacokinetic analysis of the data was performed using one-compartmental method with Phoenix WinNonlin version 1.3 (Pharsight Corp., Mountainview, CA, USA) as described in Section 3.2.3.3.

The ratios of all PK parameters between simulated microgravity (ABR) and ambulatory (AMB) groups on each subjects were calculated by the equation as below. The mean values and standard deviation of ratios on each PK parameters were derived.

AUC* = ABR (AUC)/AMB (AUC)

Cmax* = ABR (Cmax)/AMB (Cmax)

Tmax* = ABR (Tmax)/AMB (Tmax)

T1/2* = ABR (T1/2)/AMB (T1/2)

CL* = ABR (CL)/AMB (CL)

Vd* = ABR (Vd)/AMB (Vd)

* The ratio of PK parameters between tow gravity condition groups.

3.2.5.4 Statistical analysis

Scopolamine concentration data and PK parameters were reported as mean and standard deviation (SD). Differences of pharmacokinetic parameters between ambulatory and simulated microgravity conditions were tested by student's t-test, with the level of significance set at $\alpha=0.05$.

3.2.6. Compartmental pharmacokinetic modeling study

3.2.6.1 Scopolamine and SCOP-G profiles of plasma, saliva and urine

Scopolamine and SCOP-G concentration-time Profiles in plasma and saliva, respectively, were plotted with mean values of concentrations and standard deviation (SD) at each time points, respectively.

Urine data were collected as scopolamine and SCOP-G concentrations in the urine sample and the volume of the sample at the end of the collection interval. The amount of drug excreted during the collection interval were accumulated at each time point. We plot cumulative amounts of scopolamine and SCOP-G, respectively, versus time as the cumulative amount excreted versus time plot.

3.2.6.2 Partition coefficient ratio of scopolamine between saliva and plasma

The ratios of scopolamine and SCOP-G concentrations between plasma and saliva compartments were calculated to evaluate the distribution mechanism of compounds between plasma and saliva. The partition coefficient from plasma to saliva ($K_{s/p}$) was established by the ratio of $AUC_{saliva}/AUC_{plasma.}$

$$K_{s/p} = \frac{AUC(t)_{saliva}}{AUC(t)_{plasma}} = \frac{\int_0^t C_{saliva}(t)dt}{\int_0^t C_{plasma}(t)dt}$$

3.2.6.3 Pharmacokinetic modeling of INSCOP in plasma, saliva and urine

The pharmacokinetic model was developed based on the data collected in plasma, saliva and urine from 12 healthy human subjects of study in Aim 1 described in the Section 3.1.1. Initial estimates of individual compartmental PK parameters were derived using Phoenix

described in Section 3.2.1. Concentrations of SCOP and SCOP-G in plasma, saliva and urine were fitted simultaneously. Actual dosing and sampling times were used for the compartmental modeling. Model structures were described by mass balance equation with PK parameters between each compartment. Model discrimination was performed on data using Phoenix, by minimizing the Akaike Information Criteria (AIC) and by the comparison of the plots of quality (such as observed data vs. fitted data, weighted residual vs. fitted data, weighted residual vs. time).

Different compartmental PK models were tested to describe the scopolamine and its SCOP-G in plasma, saliva and urine compartments. Nonlinear and linear PK models between plasma and saliva compartments were tested for describing the relationship between scopolamine in these two compartments.

Individual estimates for pharmacokinetic parameters were assumed to follow a log-normal distribution. Therefore an exponential distribution model was used to account for intersubject variability (IIV) as follows:

$$P_i = P \cdot exp(\eta_i)$$

Where *Pi* is the individual parameter estimate for individual *i*, *P* is the typical population parameter estimate, and *ηi* was assumed to be distributed N (0, ω^2). Only significant IIVs in pharmacokinetic parameters were retained.

Residual unexplained variability was implemented as either a proportional or combined error model:

$$C_{observed,ij} = C_{pred,ij} \times (1 + \varepsilon_{p,ij}) + \varepsilon_{a,ij}$$

Where $C_{observed,ij}$ represents the observed concentration for individual *i* and observation *j*, $C_{pred,ij}$ represents the individual predicted concentration, and $\varepsilon_{p,ij}$ and $\varepsilon_{a,ij}$ represent the proportional and additive errors distributed following N (0, σ^2). Selection of an appropriate residual error model was based on the likelihood ratio test and inspection of the goodness-of-fit plots.

3.2.6.4 Model optimization

To determine the best fit pharmacokinetic model structure, model selection and identification of variability were based on minimizing the Akaike Information Criteria (AIC) of the data, achieving adequate parameter precision, inspection of goodness-of-fit plots and by comparison of the quality of fit plots.

3.2.6.5 Model validation

Model validation can be defined as the evaluation of the predictability of the developed model. Two types of validation methods were used in our study including internal validation and external validations.

Simulation was performed as the resampling technique in internal validation by Phoenix NLME, consisting of repeated random sampling with replacement from the original data (data set 1 from Aim 1). Final estimates of PK parameters, Sigma (random residual variability) and Omega (interindividual variability) from model fitting results were used in simulation. This resampling was repeated 500 times. 95% of confidence interval was obtained and the observed data were plot into 95% confidence interval (CI) profiles for plasma, saliva and urine, respectively.

External validation was performed as the application of the developed model to a new data set (data set 2) from Aim 2 study. External validation provides the most stringent method for testing a developed model. Observed values of INSCOP concentrations in plasma and saliva compartments from data set 2 were plotted into the respective 95% CI profiles from internal simulation results. Concentrations of INSCOP in saliva were used to simulate the plasma concentration of INSCOP from data set 2. Numeral check was performed by calculating prediction errors on concentrations at each time points from data set 2. The percentages changes between the prediction and observation at each time points were obtained.

Chapter 4 Results

The results of this investigation are summarized in the following four subtopics: (1) Dose escalation pharmacokinetics of intranasal scopolamine gel formulation, (2) sex impacts on pharmacokinetics of INSCOP in plasma, (3) relative bioavailability study of INSCOP in healthy human subjects under ambulatory and simulated microgravity conditions, and (4) compartmental pharmacokinetic modeling of INSCOP.

4.1. Dose escalating pharmacokinetics of INSCOP in normal human subjects

4.1.1. Validation of LC-MS/MS assay method

Concentrations of scopolamine in plasma, saliva and urine were measured by a modified LC-MS/MS method. Precision and accuracy of the assay were acceptable with r^2=0.99 or better for the linearity established by the regression of response areas across the detected concentrations range of 100 -1000 pg/mL, with an LLOQ of 50 pg/mL. Inter and intra-day coefficients of variation were below 10%. The extraction recoveries were 77.2 % for 100 pg/mL and 86.4% for 1000 pg/mL, and the coefficient of variation for percent extraction-recovery was below 5% at all concentrations.

4.1.2. Side effects results

The intranasal scopolamine formulation (INSCOP) was well tolerated with no reports of clinically significant unexpected adverse events. Vital signs were not significantly different among treatments. The most commonly reported Expected Adverse Event was

sleepiness or drowsiness which was experienced by nine of twelve subjects, and this effect was not statistically correlated with the dose. Three female subjects reported dizziness or lightheadedness within three hours after dosing of 0.4 mg. Two other female subjects reported nasal burning within 10 minutes after dosing, one after a 0.2 mg dose and another after the 0.4 mg dose. One female subject reported aftertaste within fifteen minutes of dosing. All of these minor discomforts were resolved within 30 min after dosing.

4.1.3. Plasma concentration-time profiles

A total of twelve healthy human subjects administered with three dose levels were employed in this study. Each subject had 13 time points of the plasma INSCOP concentrations. Mean plasma concentration versus time profiles for the three doses of scopolamine in 12 subjects were presented in **Figure 9**. Absorption of the drug was fast, reaching maximum plasma concentrations within one hour after dosing, and declined exponentially thereafter reaching concentrations below the LLOQ of 50 pg/mL in most subjects in 6 hours after the 0.1 mg dose, in 8 hours after the 0.2 mg dose, and 12 hours after the 0.4 mg dose. Mean plasma concentration versus time profiles for the three doses of SCOP-G in 12 subjects were presented in **Figure 10.**

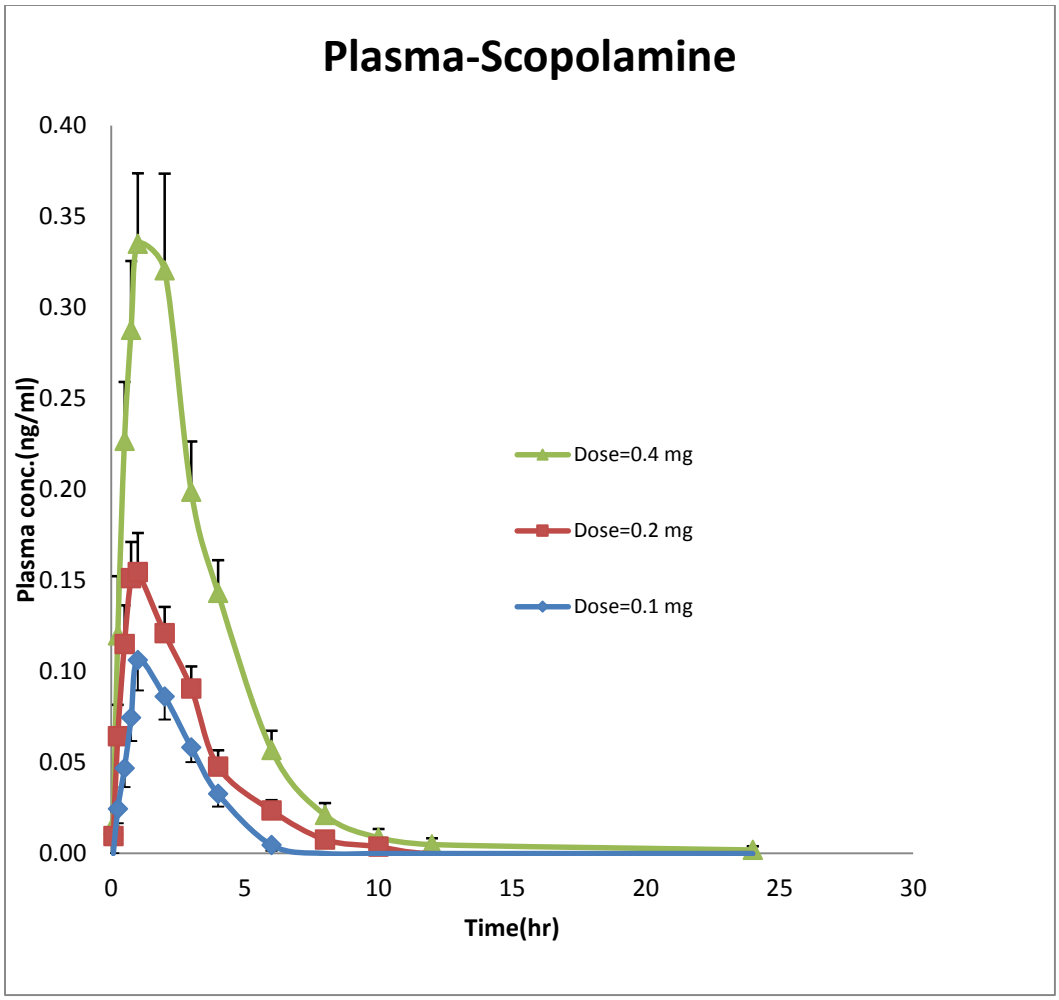


Figure 9. Mean Plasma Concentration versus Time Profile of Scopolamine after Administration of INSCOP to Normal Subjects at Three Dose Levels of 0.1 mg (♦), 0.2 mg (■), and 0.4 mg (▲). Error bars corresponded to standard deviations.

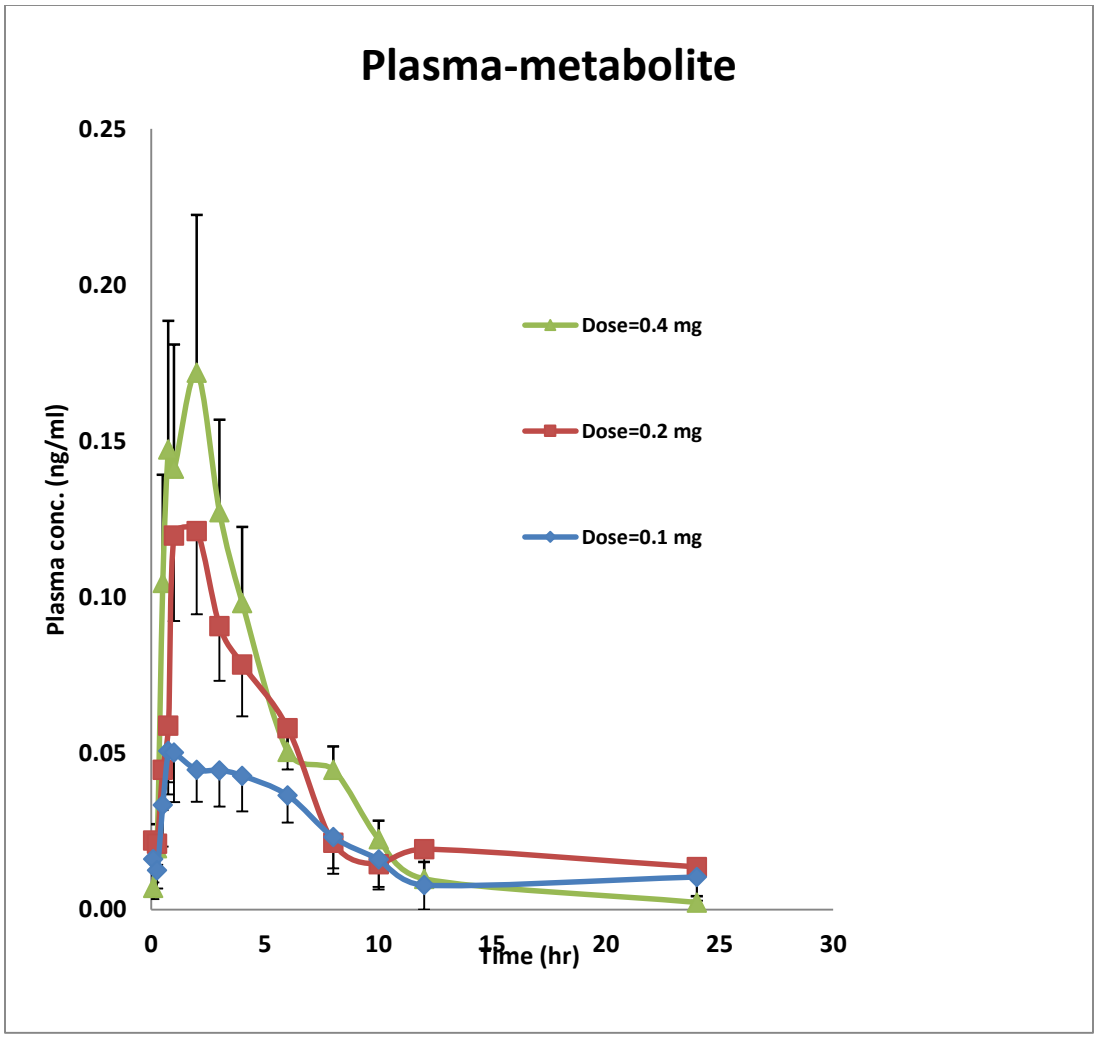


Figure 10. Mean Plasma Concentration versus Time Profile of Scopolamine Glucuronide Metabolite after Administration of INSCOP to Normal Subjects at Three Dose Levels of 0.1 mg (♦), 0.2 mg (■), and 0.4 mg (▲).Error bars corresponded to standard deviations.

4.1.4. One-compartment pharmacokinetic analysis of plasma data

Plasma concentration versus time data were fitted to a one-compartment model using Phoenix WinNonlin (version 6.3, Mountain View, CA, USA) to derive pharmacokinetic parameters. Mean values of pharmacokinetics parameters calculated for the three dose levels were presented in **Table 4**. The C_{max} of INCOP were 0.09 ± 0.04, 0.15±0.07 and 0.33 ± 0.1 ng/ml from 0.1,0.2 and 0.4 mg dose levels, respectively. The t_{max} (time to reach the C_{max}) occurred at approximately 1.2-1.3 h after the administration of all three doses in all subjects. The AUC were 0.33±0.20, 0.61±0.20 and 1.31±0.60 ng* h/ mL for three dose levels, respectively. The increases in observed C_{max}, AUC as well as the dose and body weight normalized C_{max} and AUC were compiled with doses (**Table 4**). Results also indicated that INSCOP has a short half-life (1.1h ~ 1.4h) after administration of all the three doses in all the subjects.

Table 4. Pharmacokinetic Parameters after Administration of Scopolamine at Three Dose Levels to Normal Subjects (N=12)

PK parameters (Mean±SD)	Units	Dose(mg)		
		0.1	0.2	0.4
C_{max}	(ng/mL)	0.09±0.04	0.15±0.07	0.33±0.10
(C_{max}/act dose)*(70KG/BW)	(ng/mL)	0.67±0.40	0.64±0.40	0.73±0.60
T_{max}	(hr)	1.34±0.40	1.17±0.30	1.31±0.50
Ka	(hr^{-1})	1.10±1.70	1.50±1.20	1.50±1.80
AUC	(hr*ng/mL)	0.33±0.20	0.61±0.20	1.31±0.60
(AUC/act dose)*(70KG/BW)	(hr*ng/mL)	2.71±1.20	2.51±1.80	2.97±2.87
Vd/F	(L)	627.59±351.90	1146.49±987.23	876.85±535.90
Cl/F	(L/hr)	442.93±346.10	695.28±795.20	432.04±193.20
$T_{1/2}$	(hr)	1.07±0.31	1.36±0.40	1.36±0.40

* C_{max}, maximum plasma concentration;

(C_{max}/act dose)*(70KG/BW), maximum plasma concentration normalized by administered dose and adjuted bodyweight (70KG);

T_{max}, time to reach maximum concentration;

Ka, absorption rate constant;

AUC, area under the plasma concentration-time profile curve;

(AUC/act dose)*(70KG/BW), AUC normalized with administered dose and adjusted body weight (70KG);

Vd/F, apparent volume of distribution;

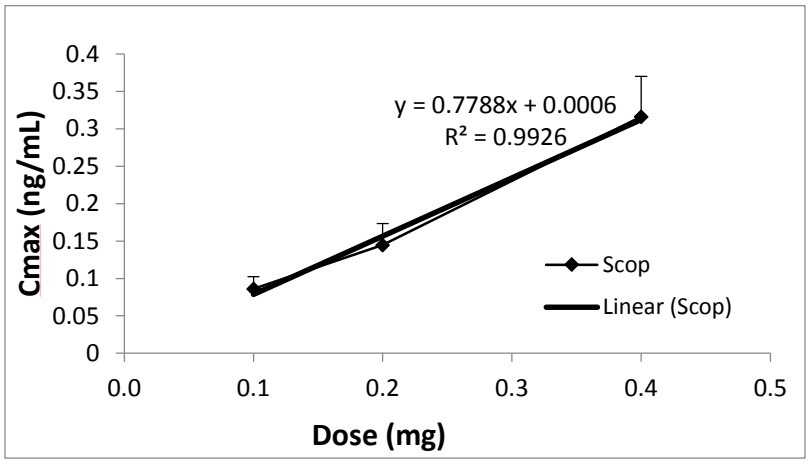
CL/F, apparent total body clearance;

T½, terminal elimination half-life; SD, standard deviation.

4.1.5. Dose linearity of INSCOP

Absorption and bioavailability appeared to be linear at the administered dose range as indicated by Cmax and AUC (**Figures 11a and 11b**). The AUC and Cmax normalized by the dose and standard body weight (**Figures 12a and 12b**) were also consistent confirming the dose linearity in PK of INSCOP at the dose range of 0.1-0.4 mg. These results indicated consistent linearity of absorption and bioavailability at the tested doses after the administration of INSCOP to normal healthy human subjects.

(a)



(b)

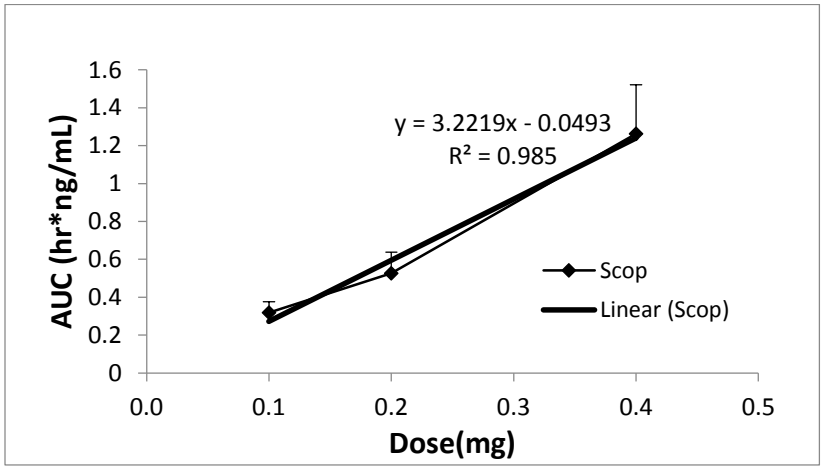
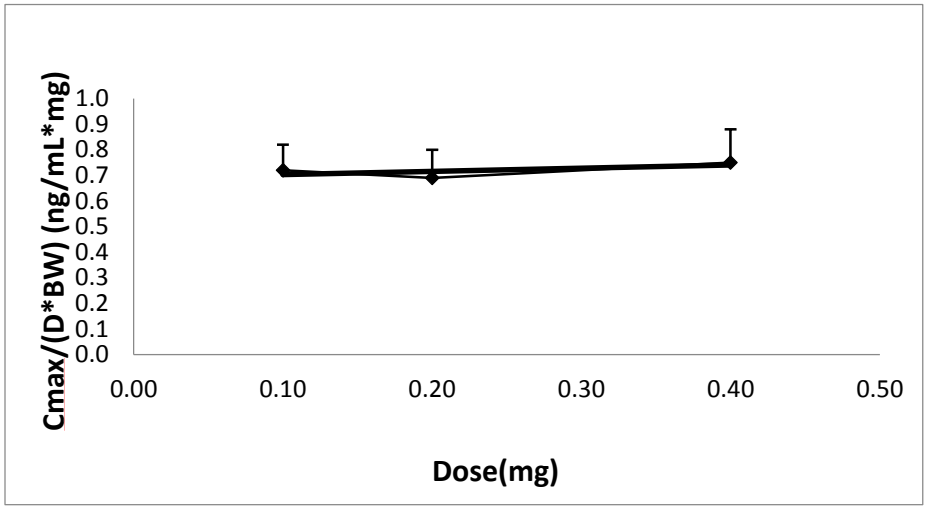


Figure 11. Dose Linearity of INSCOP Indicated by C_{max} and AUC

Error bars corresponded to standard deviations.

(a)



(b)

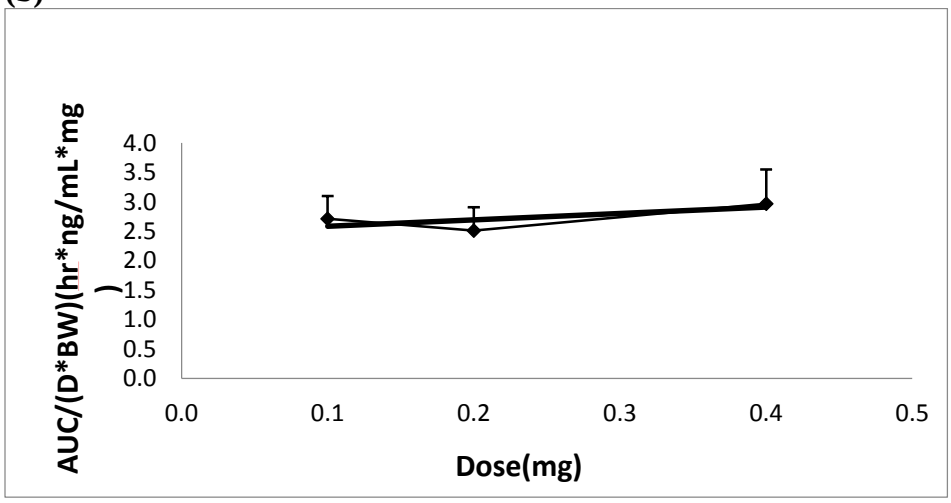


Figure 12. Dose Linearity of INSCOP Indicated by C_{max} and AUC Normalized by Dose and Standard Body Weight. Error bars corresponded to standard deviations.

4.1.6. Saliva concentration-time profiles

Twelve healthy human subjects administered with the three dose levels were employed in this study, with 13 time points of the saliva INSCOP concentrations collected in each subject. Mean saliva concentrations versus time profiles for the three doses of scopolamine in 12 subjects were presented in **Figure 13**. Absorption of the drug in saliva was fast, reaching maximum saliva concentrations within one hour after dosing, and declined exponentially after the administration of INSCOP at three doses. Saliva concentrations ranged from 0.03 to 54.8 ng/ml, 0.04 to 120.6 ng/ml and 0.04 to 182.7 ng/ml for dose of 0.1, 0.2 and 0.4 mg, respectively. The mean saliva concentrations versus time profiles scopolamine glucuronide for the three doses of scopolamine were presented in **Figure 14.**

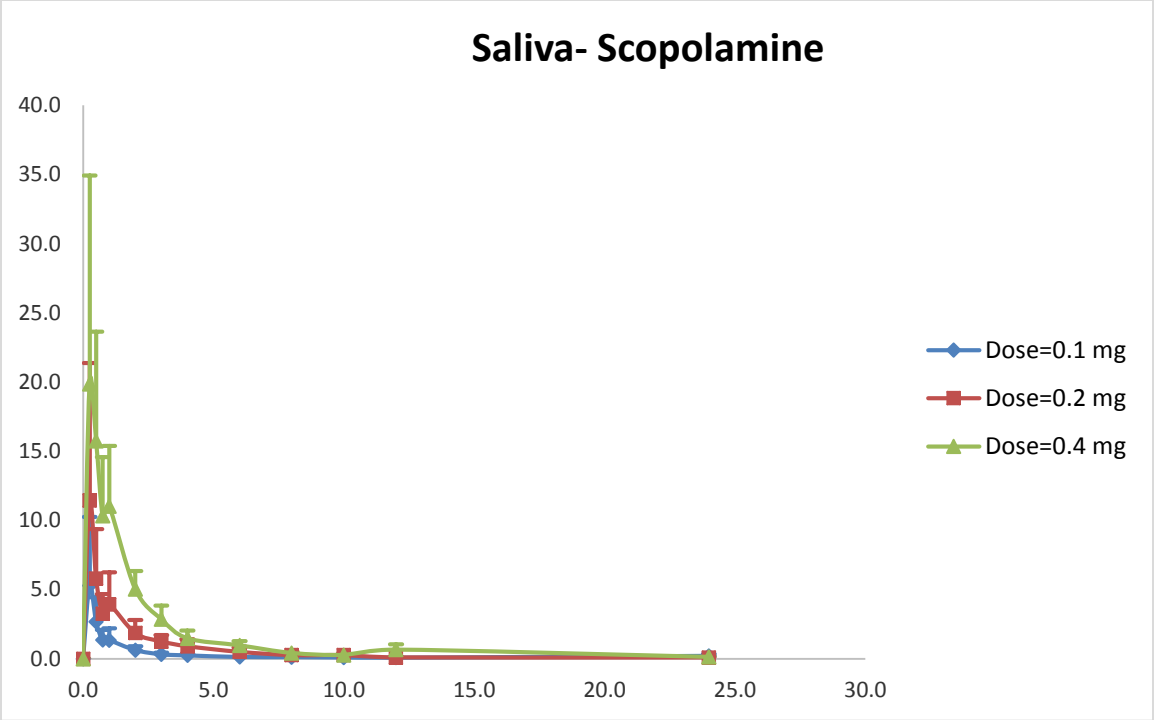


Figure 13. Mean Saliva Concentration versus Time Profile of Scopolamine after Administration of INSCOP to Normal Subjects at Three Dose Levels of 0.1 mg (♦), 0.2 mg (■), and 0.4 mg (▲).Error bars corresponded to standard deviations.

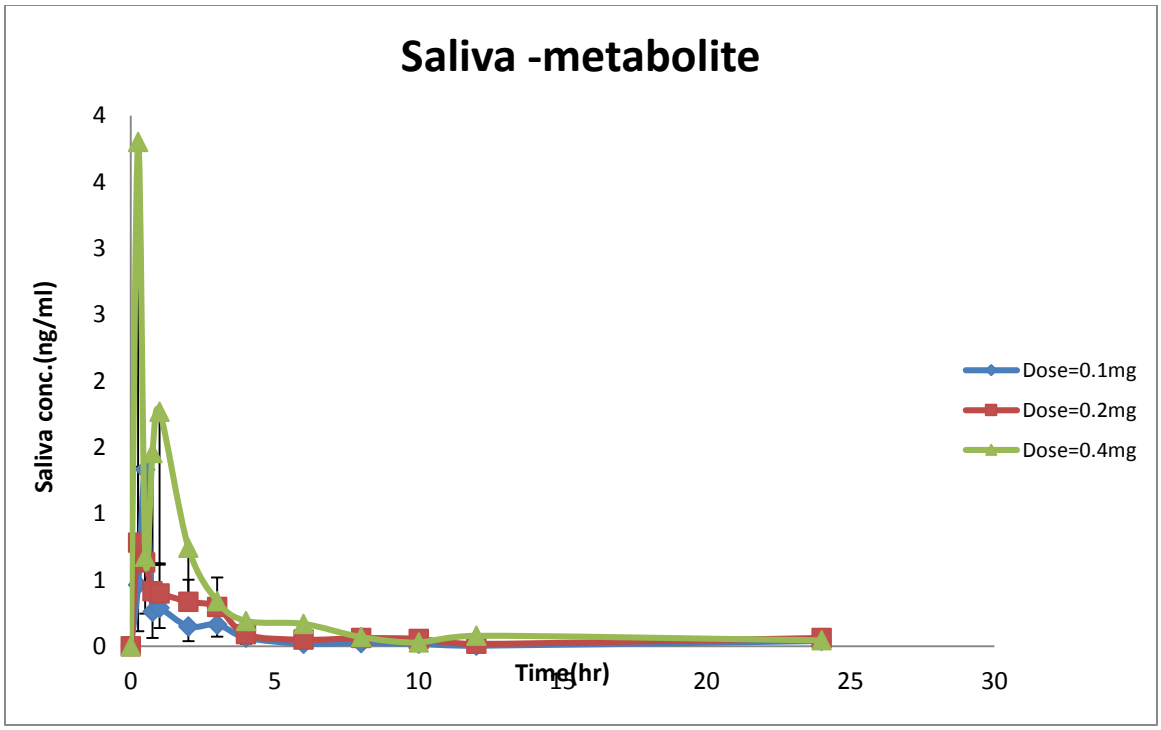


Figure 14. Mean Saliva Concentration versus Time Profile of Scopolamine Glucuronide after Administration of INSCOP to Normal Subjects at Three Dose Levels of 0.1 mg (♦), 0.2 mg (■), and 0.4 mg (▲).Error bars corresponded to standard deviations.

4.1.7. Pharmacokinetic analysis of saliva data

Data of saliva concentrations of scopolamine and SCOP-G were sequentially used for non-compartmental PK analysis (Phoenix version 6.3). The mean values of PK parameters from individual parent and SCOP-G were shown in **Table 5**. The C_{max} (Mean ±SD) of scopolamine were 10.5 ± 21.5, 13.7±35.5 and 26.9 ± 52.2 ng/ml in 0.1, 0.2 and 0.4 mg dose level, respectively. The C_{max} of SCOP-G were 0.82±1.49, 1.37±3.37and 1.03±1.81 ng/mL at the three doses, respectively. The T_{max} (time to reach the C_{max}) occurred at approximately 0.6-2.2 h after administration of all three doses in all the subjects for parent and SCOP-G compounds. The AUC were 9.9±20.5, 15.1±24.5and 32.1±34.0 ng*h/ mL for scopolamine, while the values were 1.44±1.92, 3.47±7.09 and 3.82±4.04 ng/ (mL*h) for SCOP-G at the three dose levels, respectively.

Table 5. Pharmacokinetic Parameter Values of INSCOP and SCOP-G Derived from Saliva Data after Administration of Scopolamine to Normal Subjects (N=12)

PK parameters (Mean±SD)	Saliva-Parent			Saliva-Metabolite		
	Dose (mg)			Dose (mg)		
	0.1	0.2	0.4	0.1	0.2	0.4
Cmax(ng/mL)	10.5±21.5	13.7±35.5	26.9±52.2	0.82±1.49	1.37±3.37	1.03±1.81
Tmax(hr)	0.66±0.5	1.3±1.3	1.4±1.7	1.01±0.97	0.98±0.71	2.23±1.58
AUC(hr*ng/mL)	9.9±20.5	15.1±24.5	32.1±34.0	1.44±1.92	3.47±7.09	3.82±4.04
T1/2(hr)	1.7±0.9	2.4±1.1	2.6±0.6	2.31±2.78	6.21±15.31	1.65±1.17
Vd/F	249.1±221.3	286.4±348.0	138.7±103.6	NA	NA	NA
Cl/F	88.6±64.2	97.3±194.6	32.4±24.4	NA	NA	NA

4.1.8. Urinary Excretion profiles

Urinary excretion rate profiles of scopolamine as a function of time after the administration of all three doses were presented in **Figure 15,** and separately for males and females in **Figure 16** and **Figure 17.** Urinary excretion of the drug was fast, reaching maximum excretion rate within two hours after the administration of INSCOP at three doses.

The urinary excretion rate profiles of SCOP-G as a function of time after the administration of all three doses were presented in **Figure 18,** and separately for males and females in **Figure 19** and **Figure 20.**

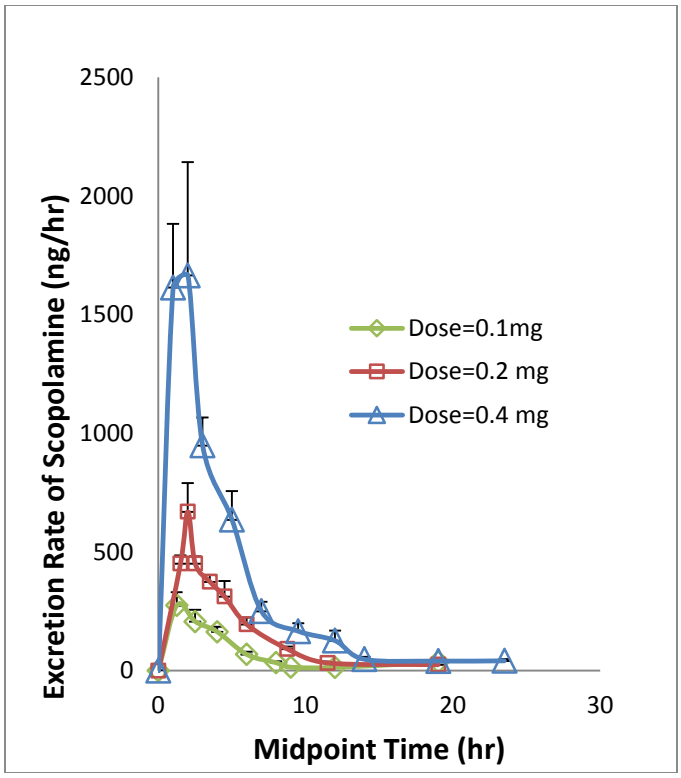


Figure 15. Mean Profile of Urinary Excretion Rates of Scopolamine at Three Doses in All Subjects (n=12). Error bars corresponded to standard deviations.

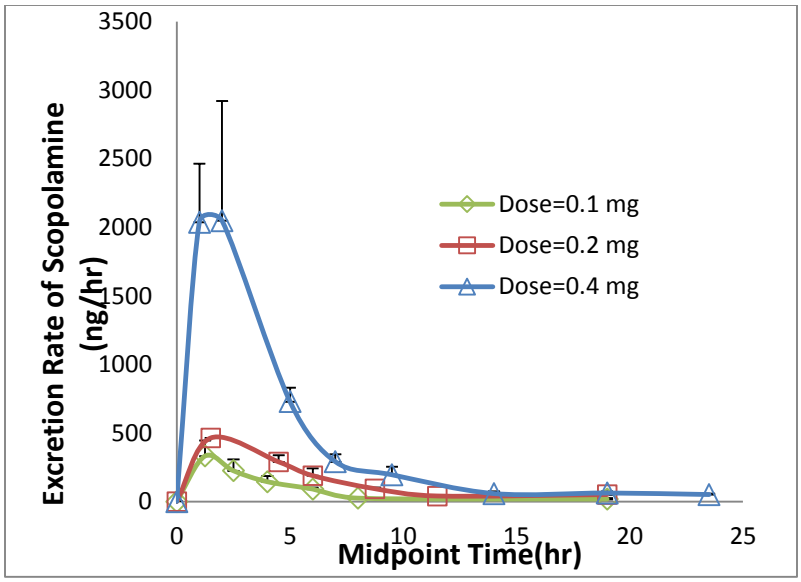


Figure 16. Mean Profile of Urinary Excretion Rates of Scopolamine at Three Doses in Male Subjects (n=6). Error bars corresponded to standard deviations.

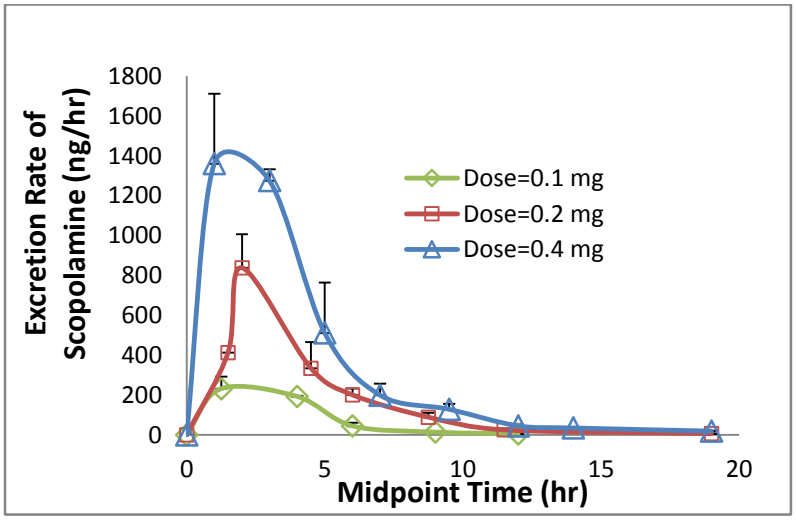


Figure 17. Mean Profile of Urinary Excretion Rates of Scopolamine at Three Doses in Female Subjects (n=6). Error bars corresponded to standard deviations.

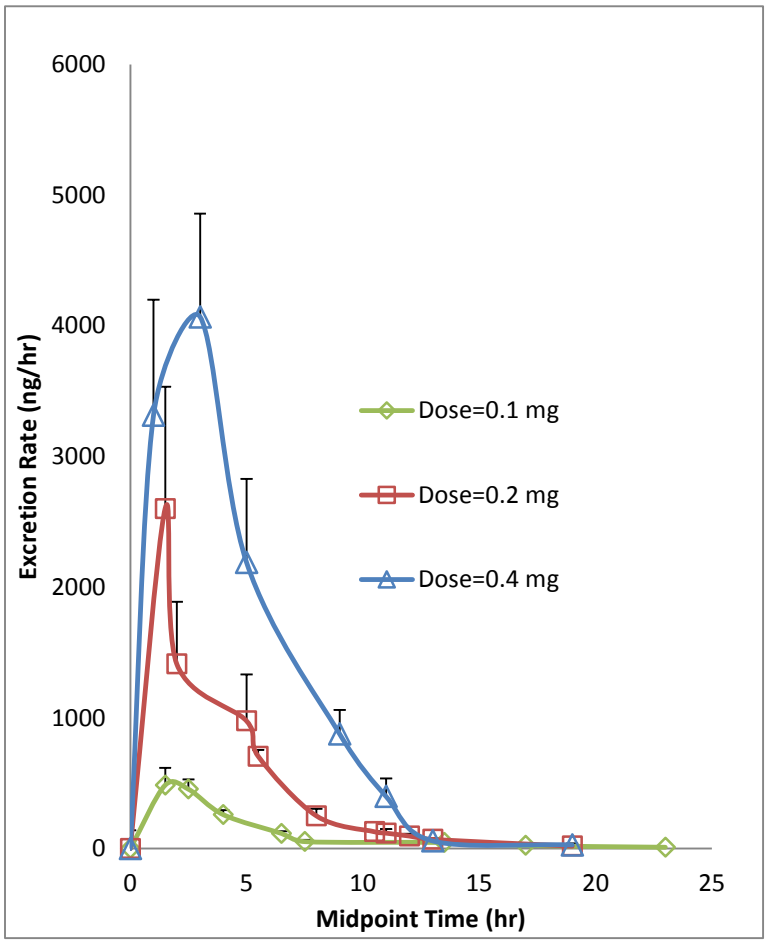


Figure 18. Mean Profile of Urinary Excretion Rates of SCOP-G at Three Doses in All Subjects (n=12). Error bars corresponded to standard deviations.

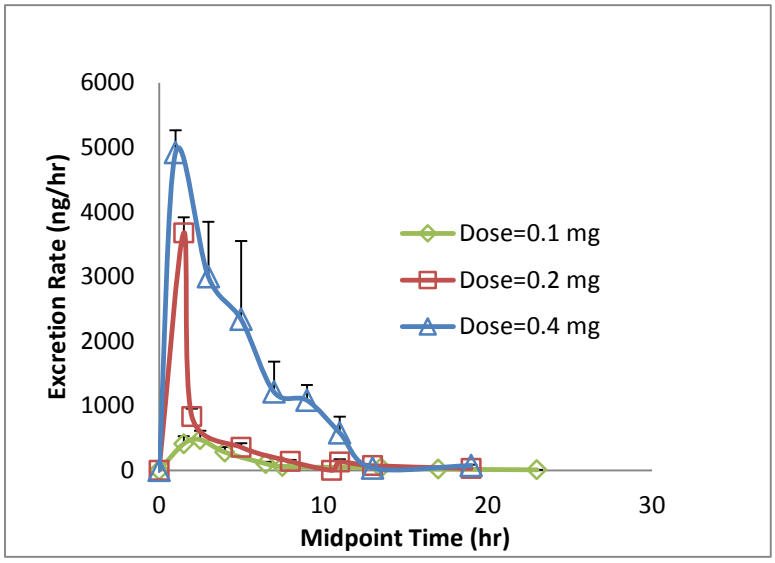


Figure 19. Mean Profile of Urinary Excretion Rates of SCOP-G at Three Doses in Male Subjects (n=6). Error bars corresponded to standard deviations.

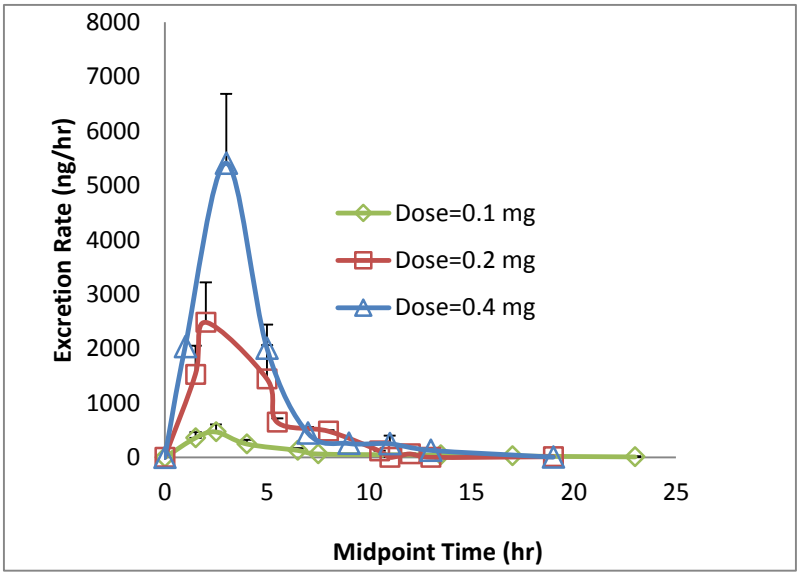


Figure 20. Mean Profile of Urinary Excretion Rates of SCOP-G at Three Doses in Female Subjects (n=6). Error bars corresponded to standard deviations.

4.1.9. Non-compartment pharmacokinetic analysis of urinary data

Urinary excretion data of scopolamine and SCOP-G were analyzed by non-compartmental PK analysis (Phoenix version 6.3). The PK parameters of parent (SCOP) and SCOP-G (scopolamine glucuronide) from urinary excretion data after INSCOP administration to normal subjects were shown in **Table 6** and **Table 7**.

The percentage of dose excreted in the urine as scopolamine was consistent for all three doses, with less than 1.5 % of the administered dose being eliminated unchanged confirming that hepatic metabolism, not renal excretion is the major pathway for the elimination of scopolamine in humans. However, the percentage of dose excreted as scopolamine glucuronide, a significant SCOP-G excreted in the urine, was less than 5.2% at all doses, suggesting the existence of other metabolites from hepatic metabolism and/or from extra-hepatic pathways of metabolism of scopolamine in humans. The total amount of scopolamine excreted in the urine (Xu) of INCOP were 1.44 ± 0.17, 3.19±0.47 and 6.29 ± 0.70 µg from 0.1,0.2 and 0.4 mg dose levels, respectively; while the Xu values were 4.49 ± 0.46, 11.20±2.17 and 20.80 ± 2.83 µg for scopolamine glucuronide metabolite. The area under the urinary excretion rate-time curve (AUXu) were 1.29 ± 0.18, 2.88±0.46 and 6.14 ± 0.74 mg*h/mL for scopolamine, and 4.22 ± 0.40, 10.55±2.08 and 21.30 ± 2.74 mg*h/mL for SCOP-G at 0.1, 0.2 and 0.4 mg dose levels, respectively. The results of fraction excreted unchanged in urine were consistent for scopolamine at the three doses, which was less than 1.5%.

After statistical analysis of PK parameters between males and female, no significant differences were found on all PK parameters at the three dose levels, which indicated there was no sex differences on elimination of INSCOP.

Table 6. Pharmacokinetics Parameters of Scopolamine Derived from Urinary Excretion of Scopolamine after INSCOP Administration to Normal Subjects

Parameters	Unit	Dose(mg)								
Mean±SD		0.10			0.20			0.40		
		All	female	male	All	female	male	All	female	male
N		12	6	6	12	6	6	12	6	6
Xu	μg	1.44±0.17	1.5±0.2	1.38±0.30	3.19±0.47	3.46±0.80	2.92±0.40	6.29±0.70	6.41±0.8	6.18±1.2
Percent_Recovered	%	1.25±0.22	1.18±0.29	1.32±0.41	1.43±0.27	1.46±0.48	1.40±0.30	1.36±0.18	1.49±0.27	1.24±0.25
AUXu	hr*mg/mL	1.29±0.18	1.31±0.27	1.27±0.28	2.88±0.46	3.10±0.87	2.67±0.42	6.14±0.74	6.43±0.85	5.85±0.73
T1/2	hr	2.96±0.52	2.32±0.40	3.59±1.2	3.62±0.75	2.34±0.52	4.91±1.4	3.42±0.40	2.9±0.35	3.94±0.77
K	/hr	0.30±0.04	0.32±0.05	0.27±0.08	0.29±0.06	0.38±0.09	0.19±0.04	0.23±0.02	0.25±0.07	0.20±0.04
fe		0.013±0.003	0.015±0.002	0.011±0.002	0.015±0.003	0.016±0.004	0.014±0.002	0.015±0.002	0.016±0.002	0.014±0.003

*Xu, total amount of scopolamine excreted in the urine;

AUXu, area under the urinary excretion rate-time curve;

T½, terminal elimination half-life;

K, elimination rate constant;

fe, fraction excreted unchanged in urine;

SD, standard deviation.

Table 7. Mean Values of Pharmacokinetic Parameters Derived from Urinary Excretion of Scopolamine Glucuronide after INSCOP Administration to Normal Subjects

Parameters	Unit	Dose(mg)								
Mean±SD		0.1			0.2			0.4		
		All	female	male	All	female	male	All	female	male
N		12	6	6	12	6	6	12	6	6
Xu	μg	4.49±0.46	4.50±0.90	4.48±1.0	11.20±2.17	14.3±3.80	8.12±3.19	20.80±2.83	19.3±1.80	22.62±6.20
Percent_Recovered	%	4.49±0.46	4.48±0.90	4.50±1.0	4.21±1.16	4.95±1.80	3.46±1.45	5.20±0.71	4.82±0.45	5.60±1.54
AUXu	hr*mg/mL	4.22±0.40	4.95±1.0	3.49±0.7	10.55±2.08	10.94±2.60	10.16±1.90	21.30±2.74	20.50±2.10	22.10±2.90
T1/2	hr	2.76±0.53	2.0±0.10	3.52±0.85	2.21±0.61	1.49±0.39	2.93±1.36	1.69±0.21	1.52±0.11	1.86±0.46

*Xu, total amount of scopolamine excreted in the urine;

AUXu, area under the urinary excretion rate-time curve;

T½, terminal elimination half-life;

SD, standard deviation.

4.2. Sex impact on PK of INSCOP in plasma

In dose escalating study of INSCOP, a total of 12 healthy human subjects were employed in the study, including 6 males and 6 females. The PK parameters of INSCOP for males and females at the three dose levels were derived and found the sex differences on several PK parameters. In this part of study, we used the plasma time profiles as well as three covariates including sex, body weight, and age, to study the covariate impacts on the INSCOP pharmacokinetics.

4.2.1. Subject demographics and actual dosing

Twelve healthy (data set 1), non-smoking human subjects between 21 and 47 years of age with matching astronaut's age group, participated in the study. All subjects had normal values for hepatic and renal functions (albumin, ALT, ALP, AST, bilirubin, BUN, hematocrit, GGT, and urinary creatinine clearance), as well as indices of cardiovascular function (blood pressure and pulse rate). Mean values of subject information are presented in **Table 8**. Details of subject demographics and actual dosing are presented in **Table 9**.

Table 8. Demographics of Study Subjects in Data Set 1

Gender	Male	Female
N	6	6
Age (Mean ± SD)	37 ± 8.5	29 ± 5.4
Body weight (Mean ± SD)	88.7 ± 9.6	72.8 ± 14.3
Average BMI	26	

* SD, standard deviation; BMI, body mass index.

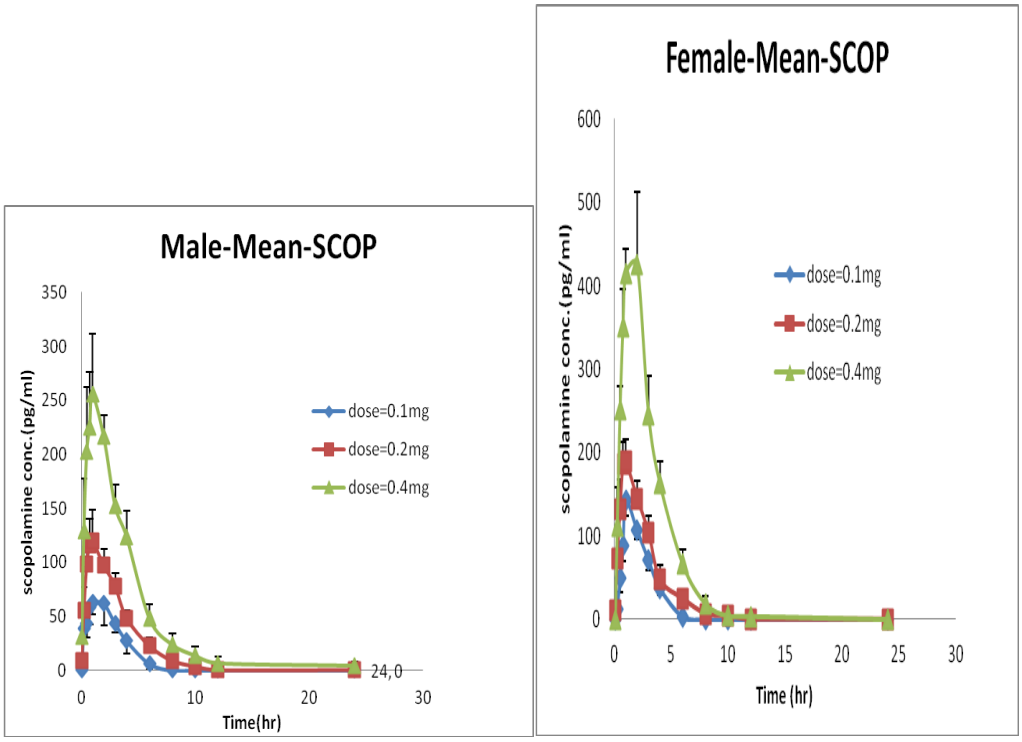
Table 9. Demographics and Dosing Data of Individuals

Subject	Gender	Age	Weight(Kg)	Height(cm)	Target Dose(mg)		
					0.1	0.2	0.4
					Delivered Dose(mg)		
1	F	21	70.8	168	0.105	0.208	0.416
2	F	34	51.3	160	0.276	0.216	0.31
3	F	25	81.2	170	0.102	0.525	0.43
4	F	33	83	168	0.103	0.183	1.01
5	F	33	61.7	160	0.108	0.492	0.35
6	F	26	88.9	178	0.289	0.148	0.402
7	M	38	71.2	173	0.049	0.168	1.194
8	M	47	89.4	185	0.1	0.164	0.348
9	M	42	98	196	NA	0.57	0.4
10	M	39	96.6	191	0.235	0.202	0.298
11	M	35	86.6	180	0.109	0.215	0.426
12	M	22	90.3	178	0.107	0.214	0.87
Mean		32.92	80.75	175.58	0.14	0.28	0.54
SD		8.1	14.27	11.33	0.08	0.16	0.3

4.2.2. Plasma concentration-time profiles of males and females

A total of 12 healthy human subjects were employed in this study, including 6 males and 6 females. The mean (± SD) plasma scopolamine concentration-time profiles in males and females are presented in **Figure 21**.

The scopolamine concentration profiles in females were higher than those in males at the three dose levels. After statistical analysis, mean maximum plasma concentration (Cmax) of scopolamine in females was significantly higher than that in males ($p<0.05$). The absorption and distribution phases were similar between male and female at the three dose levels.



(a) (b)

Figure 21. (a) Mean Plasma Concentration-time Profiles of scopolamine at Three Dose Levels for 6 Male Subjects, (b) Mean Plasma Concentration-time Profiles of scopolamine at Three Dose Levels for 6 Female Subjects [Dose = 0.1 mg (♦), Dose = 0.2 mg (■), Dose = 0.4 mg (▲)]. Error bars corresponded to standard deviations.

4.2.3. Individual pharmacokinetic analysis for males and females

Plasma concentration versus time data were fitted to a one-compartment model using Phoenix WinNonlin (version 6.3, Mountain View, CA, USA) to derive pharmacokinetic parameters. Mean values of pharmacokinetics parameters for males and females calculated for the three dose levels are presented in **Table 10**. These data suggested that there were no significant differences between sexes after dose of 0.1 mg and 0.2 mg. However, after the administration of 0.4 mg dose, mean maximum plasma concentration (Cmax) of scopolamine in males was lower than that in females ($p<0.05$), resulting from a faster clearance and larger volume of distribution in males than in females ($p<0.05$).

Table 10. Pharmacokinetics Parameters in Male and Female Subjects after Administration of INSCOP at Three Dose Levels.

Parameters (Mean±SD)	Unit	Dose (0.1 mg)		Dose (0.2mg)		Dose (0.4mg)	
		female	male	female	male	female	male
N		6	6	6	6	6	6
Cmax	(ng/mL)	0.1±0.02	0.1±0.02	0.2±0.07	0.1±0.05	0.4±0.07*	0.3±0.1
(Cmax/act dose)*(70KG/BW)	(ng/mL)	0.8±0.4	0.5±0.3	0.8±0.5	0.5±0.3	1.1±0.7*	0.4±0.2
Tmax	(hr)	1.4±0.3	1.3±0.8	1.1±0.3	1.2±0.2	1.4±0.4	1.3±0.6
Ka	(hr^{-1})	0.7±0.2	1.4±1.3	1.5±0.9	1.5±0.8	1.0±0.7	2.1±1.7
AUC	(hr*ng/mL)	0.4±0.1	0.3±0.1	0.6±0.3	0.5±0.2	1.6±0.6	1.1±0.4
(AUC/act dose)*(70KG/BW)	(hr*ng/mL)	3.1±1.3	1.8±0.8	2.8±2.3	1.9±1.1	4.3±3.8	1.6±0.6
Vd/F	(L)	514.3±425.2	763.5±80.2	851.2±554.3	1441.8±1246.7	500.0±266.2*	1253.7±457.0
CL/F	(L/hr)	412.6±438.6	479.4±144.2	570.8±450.8	819.8±1048.5	322.8±157.2*	589.4±88.2
T1/2	(hr)	1.0±0.2	1.2±0.3	1.1±0.3	1.6±0.5	1.1±0.2	1.6±0.4

Statistically significant difference between females and males with the same dose by using a non-parametric Wilcoxon-Mann-Whitney test, with the level of significance set at $\alpha=0.05$. N=6 per group.

** AUC, area under the plasma concentration-time curve;

(AUC/act dose)*(70KG/BW), area under the curve normalized by the actual dose and adjusted to the value of 70KG bodyweight;

Cmax, maximum plasma concentration;

(Cmax/act dose)*(70KG/BW) maximum plasma concentration normalized by actual dose and adjusted to the value of 70KG bodyweight;

Tmax, time to reach maximum concentration;

T½, terminal elimination half-life;

Ka, absorption rate constant;

Vd/F, apparent volume of distribution;

CL/F, apparent total body clearance; SD, standard deviation

4.2.4. Population PK analysis

Our previous study showed the sex differences in PK parameters of scopolamine at the high dose level of 0.4 mg. Since information is lacking for the possibilities of covariates that may influence the scopolamine PK parameters, we determined the final population covariates PK model for INSCOP and characterize the population PK of scopolamine at dose level of 0.4 mg.

4.2.4.1 Base model of population PK analysis

Base on the semi-log plot of INSCOP plasma concentration versus time profile (**Figure 22**), One-compartment pharmacokinetic models with the first-order elimination was selected.

The base model structure was described as follows:

Ka= tvKa*exp(nKa)

V=tvV*exp(nV)

CL=tvCL*exp(nCL)

PK parameter estimates of absorption rate constant (Ka), volume of distribution (V), and elimination clearance (CL) were 1.0±0.03 hr^{-1}, 4.6±0.59 L/kg and 4.7±0.57 L/ (hr*kg). Model goodness of fit plot was indicated as observed concentrations versus population predicted concentrations plot (**Figure 23**). Diagnostic plot of population weighted residuals versus time did show signs of significant bias (**Figure 24**).

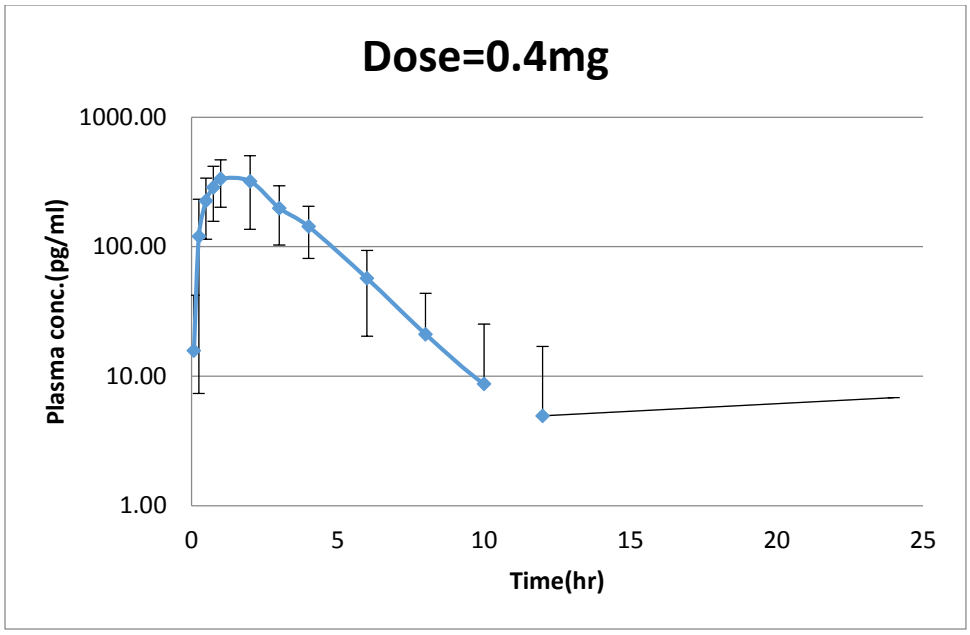


Figure 22. Semilog Profiles of Mean Scopolamine at 0.4mg Dose Levels for 12 Subjects. Error bars corresponded to standard deviations.

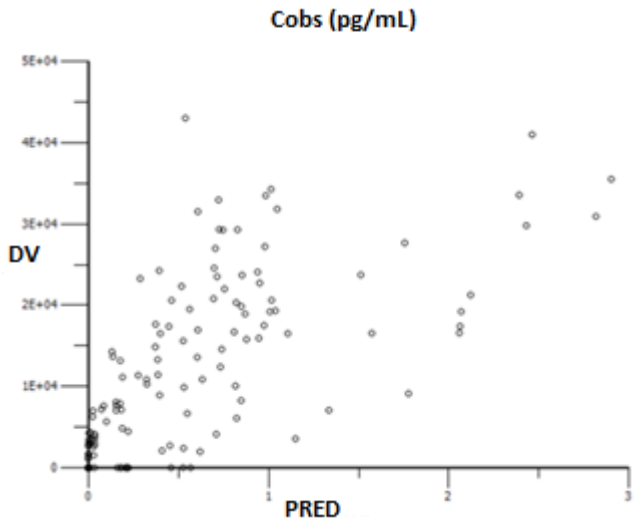


Figure 23. Goodness of Fit Plot of Base Model (DV – Observed Concentration, PRED-Population Predicted Concentration)

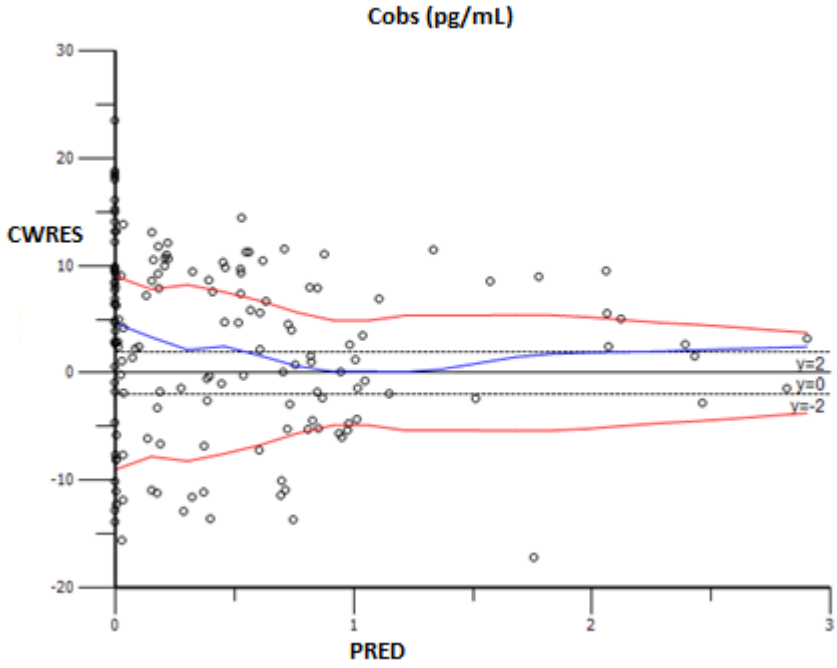


Figure 24. Weighted Residuals VS. Prediction Plots for Base Population Model

4.2.4.2 Covariance population PK analysis

After forward and backward stepwise modeling building approaches, covariates including sex, body weight and age were screened for their possible influences on scopolamine PK parameters. In the forward stepwise modeling building approach, the inclusion of sex- V, sex- Cl reduce the -2LL >3.84. In the backward elimination step, no covariates were found to increase the -2LL >3.84. Overall results of Criteria for SEX included covariate model were shown in **Table 11.**

Final covariate model for INSCOP is described as below:

Ka = tvKa * exp(nKa)

V = tvV * exp(dVdSEX1*(SEX==1)) * exp(nV)

Cl = tvCl * exp(dCldSEX1*(SEX==1)) * exp(nCl)

PK parameter estimates of absorption rate constant (Ka), volume of distribution (V), elimination clearance (CL) were 0.97±0.03 hr^{-1}, 6.8±1.0 L/kg and 5.3±0.7 L/ (hr*kg). The model goodness of fit plot were indicated as observed concentrations versus population predicted concentrations plot (**Figure 25**). Diagnostic plot of population weighted residuals versus time did show signs of significant bias (**Figure 26**). From comparison of goodness of fit plot of base model (Figure 23) and final population model, there was a significant improvement of correlation between observed and predicted concentration of scopolamine after final population analysis. The range of weighted residuals also decreased (from (-10 ~10) to (-2 ~2)). The results indicated that the sex has a significant influence on the PK of scopolamine.

Table 11. Overall Results of Criteria for SEX included Covariate Model

Scenario	LogLik	-2LL	Δ-2LL	AIC	BIC
cstep00	-824.144	1648.289	NA	1669.289	1690.638
cstep01 Ka-sex	-823.771	1647.542	0.747<3.84	1663.542	1687.941
cstep02 V-sex	-799.604	1599.208	49.080>3.84	1615.208	1639.607
cstep04 V-wt	-823.63	1647.26	1.029<3.84	1658.26	1682.658
cstep60 Cl-sex	-794.607	1589.214	59.049>3.84	1605.471	1627.198
cstep64 Cl-sex V-age	-787.461	1574.922	70.367>3.84	1590.922	1615.321
cstep66 V-sex Cl-sex	-786.986	1573.973	74.316>3.84	1591.973	1619.422

* -2LL: Minus twice the log of the likelihood;

Δ-2LL: the difference of -2LL between the steps and step00;

AIC: Akaike Information Criterion;

BIC: Bayesian Information Criterion.

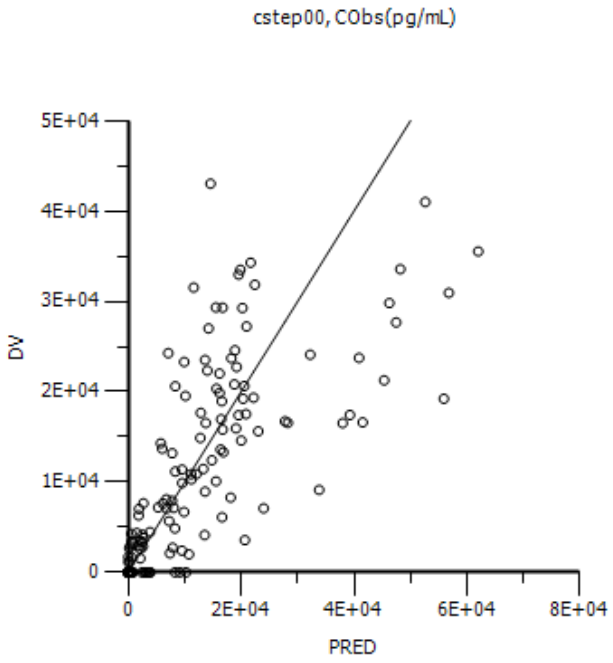


Figure 25. Goodness of Fit Plot of Final Covariate Population Model (DV – Observed Concentration, PRED-Population Predicted Concentration)

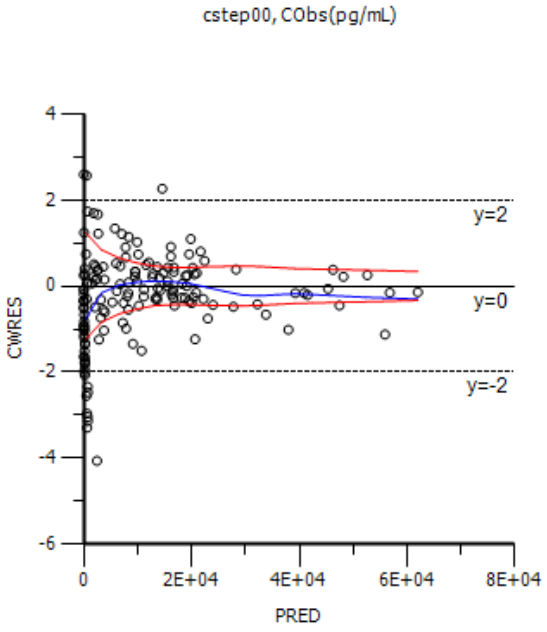


Figure 26. Population Weighted Residuals VS. Prediction Plots for Final Population Model

4.2.4.3 Comparison of PK parameters from individual and population PK analysis

The PK parameters derived from individual PK analysis and population analysis were compared in **Table 12**. After the statistical analysis by a non-parametric method of absorption rate constant (Ka), volume distribution (V) and systemic clearance (CL) between sexes, the P values were 0.18, 0.03 and 0.03, respectively.

From the population analysis, the clearance of the parent scopolamine was significantly faster and the volume of distribution was significantly higher in males than in females, As a result, including sex as a covariate to the pharmacokinetic model of scopolamine offers the best fit for PK modeling of the drug at dose of 0.4 mg.

Table 12. Prediction of PK Parameters from Base Model and Sex Covariate Model Comparing with Individual Analysis.

	Population Pharmacokinetics			Individual Estimation		
Parameters	Dose=0.4 mg					
(Mean±SE)	Unit	Base model	Covariate model	Male	Female	P value
N		12	6 Male/6 Female	6	6	
-2LL		1648	1574 (Δ-2LL=74>3.84)			<0.05
AIC		1669	1592			
tvKa	/hr	1.0±0.03	0.97±0.03	2.09±1.75	0.99±0.68	0.18
tvV	L/kg	4.6±0.59	6.8±1.0	14.77±7.33	6.72±3.03	0.03
tvCl	L/(hr*kg)	4.7±0.57	5.3±0.7	6.78±1.68	4.29±1.68	0.03
dCldSEX1		NA	-0.26			
dVdSEX1		NA	-0.52			

* N: subjects number;

-2LL: minus twice the log of the likelihood;

Δ-2LL: the difference of -2LL between the steps and step00;

AIC: Akaike Information Criterion;

tvV: typical value of volume distribution;

tvCl: typical value of clearance;

dCldSEX1: the factor of sex influence in clearance;

dVdSEX1: the factor of sex influence in volume of distribution.

4.3. Relative bioavailability study in healthy human subjects under ambulatory and simulated microgravity conditions

Plasma pharmacokinetics of INSCOP was studied in twelve healthy human subjects. The objective of this aim was to estimate the impact of simulated microgravity, antiorthostatic bed rest (ABR) condition it altered the bioavailability of scopolamine at two dose levels (0.2 mg and 0.4 mg).Anti-orthostatic bed rest (ABR) model has been used for ground-based simulation of microgravity. Basically, the subjects were made to rest in a 6-12° head-down tilt during the whole study time. We examined the pharmacokinetics in twelve healthy male and female subjects after the administration of two doses (0.2 and 0.4 mg) of INSCOP at two different gravity conditions using a four-way crossover design. Concentrations of scopolamine in plasma were measured by a validated LC-MS/MS method. Pharmacokinetic analysis was performed using WinNonlin (version 3.3) to obtain pharmacokinetic parameters. We hypothesize that there were differences of bioavailability and PK parameters between the ground and microgravity environments.

4.3.1. Subject demographics and actual dosing

The clinical protocol was reviewed and approved by the NASA Johnson Space Center Committee for the Protection of Human Subjects and the Investigational Review Board of MDS Pharma Services (Lincoln, Nebraska) where this IND clinical protocol was implemented. Twelve healthy (Data Set 2), non-smoking human subjects between 21 and 49 years of age participated in the randomized, four way crossover clinical study to evaluate and compare the pharmacokinetics of INSCOP after a single dose in simulated microgravity and in normal gravity with a seven-day washout period between treatments. Details of subject demographics and actual dosing were presented in **Table 13.**

Table 13. Demographics of Study Subjects in Data Set 2.

SUBJECT POPULATION DEMOGRAPHIC PARAMETERS		N	%*
GENDER			
	Male	6	50
	Female	6	50
AGE GROUP			
	< 20 yrs	0	0
	20 – 29 yrs	6	50
	30 – 39 yrs	3	25
	40 – 49 yrs	3	25
	50-59	0	0
RACE			
	Caucasian	11	91.7
	Non – Hispanic Black	0	0
	Hispanic	1	8.3
	Other	0	0

*Demographic percentages are calculated out of the number of subjects completed in the Study

4.3.2. Plasma concentration-time profiles

For all of twelve healthy human subjects administered with two dose levels, 13 time points of the plasma were collected for each subject at each dose level. Mean plasma concentration versus time profiles for the two doses of scopolamine were generated by calculating the mean concentration from 12 subjects at each time point. The mean (± SD) plasma scopolamine concentration-time profiles at ambulatory and simulated microgravity conditions at 0.2 mg and 0.4 mg dose levels were presented in **Figure 27.** The mean (± SD) plasma SCOP-G concentration-time profiles at ambulatory condition at 0.2 mg and 0.4 mg dose are presented in **Figure 28.**

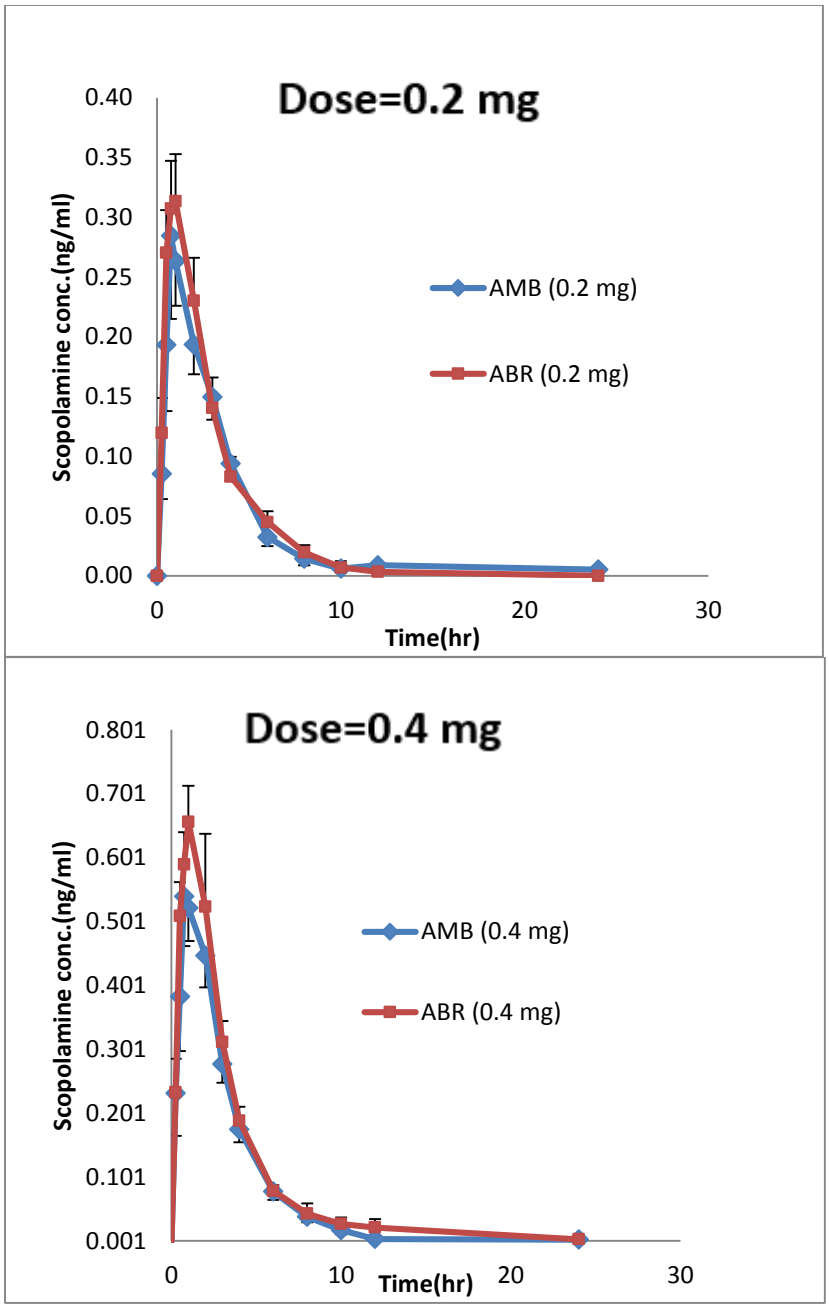


Figure 27. Linear Mean Plasma Concentration-Time Profiles of Scopolamine under AMB and ABR Conditions at 0.2mg & 0.4mg Doses. Error bars corresponded to standard deviations.

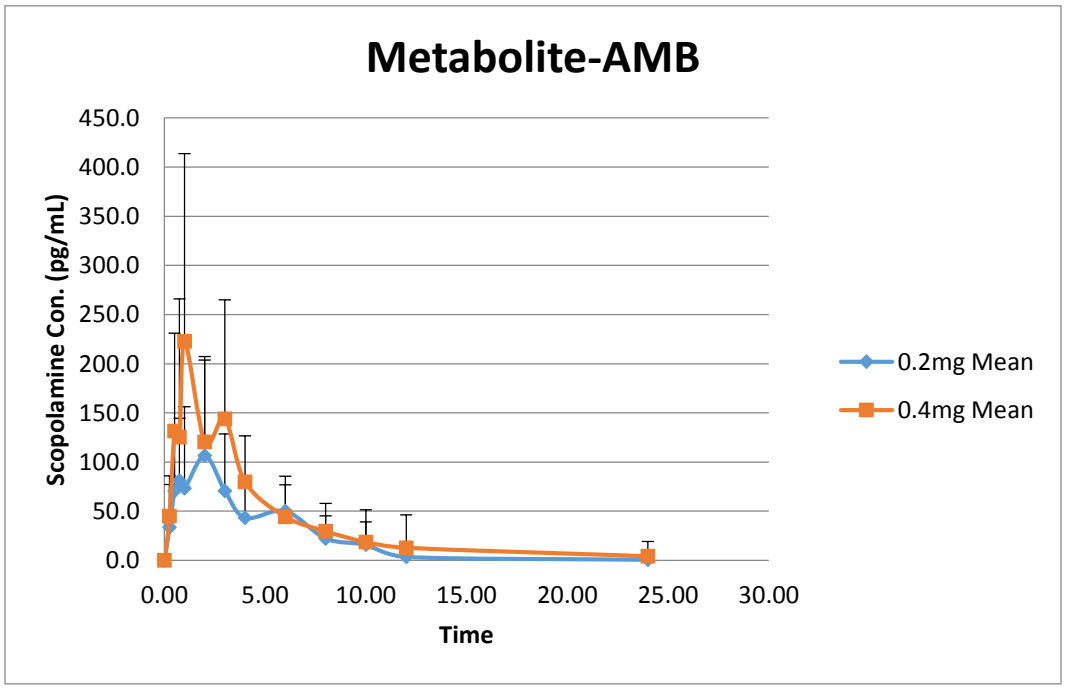


Figure 28. Linear Mean Plasma Concentration-Time Profiles of SCOP-G under AMB Conditions at 0.2mg & 0.4mg Doses. Error bars corresponded to standard deviations.

4.3.3. One-compartmental pharmacokinetic analysis of plasma data

We examined the differences of pharmacokinetics in twelve healthy, 6 males and 6 females, subjects after administration of two doses (0.2 and 0.4 mg) of the IND formulation of INSCOP, at ambulation (AMB) and simulated microgravity (ABR) conditions. Concentrations of SCOP were measured in plasma by a validated LC-MS/MS method. Pharmacokinetic analysis was performed using Phoenix WinNonlin (version 6.2) to derive pharmacokinetic parameters (Gibaldi & Perrier, 1982). Pharmacokinetic parameters were tested using SAS proc glm (Cary, NC, USA). A customary levels of significance of α=0.05 for significance testing was used. The mean values of pharmacokinetic parameters from 12 subjects were showed in **Table14**. The INSCOP pharmacokinetic parameters such as C_{max}, AUC, $t_{1/2}$ and CL/F were comparable between the two different gravity groups at the same dose level. For example, C_{max} of INCOP were 0.27±0.16 and 0.31±0.13 ng/mL in AMB and ABR groups, respectively, at 0.2 mg dose, while the values were 0.48±0.11 and 0.64±0.22 ng/ml for two gravity groups, respectively, at 0.4 mg dose. The t_{max} (time to reach the C_{max}) occurred at approximately 1.0-1.3 h after the administration of the two doses in all the subjects, which would offer the rapid onset of scopolamine. No differences on all pharmacokinetic parameters were statistically significant (student's t-test) between ambulatory and simulated microgravity conditions after administration of INSCOP with the same dose.

Mean values of pharmacokinetics parameters for males and females under ambulatory gravity condition were calculated for the two dose levels are presented in **Table 15**. These data suggested that there were no significant differences between sexes after dose of 0.2 mg and 0.4 mg.

We also calculated the ratios of all PK parameters between ambulatory and simulated microgravity conditions for each subjects, the mean values and standard deviation of the

ratios on each PK parameters were presented in **Table 16**. The ratios of C_{max} were 1.60±1.12 and 1.36±0.30 for 0.2 and 0.4 mg doses, respectively, while the values were 1.35±0.98 and 1.26±0.45 for AUC after the 0.2 and 0.4 mg dose of INSCOP administration, respectively. The results indicated that the simulated microgravity, antiorthostatic bed rest (ABR) condition does not alter the pharmacokinetics of INSCOP at the two dose levels (0.2 mg and 0.4 mg).

Table 14. Pharmacokinetic Parameters of Intranasal Scopolamine at Two Doses (0.2 and 0.4 mg) in Ambulation (AMB) and Simulated Microgravity (ABR) Conditions

PK parameters (Mean±SD) (%CV)	Dose (mg)			
	0.2		0.4	
	AMB	ABR	AMB	ABR
C_{max} (ng/mL)	0.27±0.16	0.31±0.13	0.48±0.11	0.64±0.22
	60.81	42.87	23.77	33.83
C_{max}/dose	1.45±0.73	1.50±0.6	1.07±0.47	1.74±0.51
	50.04	39.87	43.92	29.12
T_{max} (hr)	1.29±0.45	1.00±0.25	1.29±0.38	1.07±0.29
	34.85	24.42	29.79	27.51
K_a (hr^{-1})	1.17±0.73	1.73±1.38	0.97±0.41	1.61±1.32
	62.47	79.81	41.75	82.04
AUC (hr*ng/mL)	0.91±0.43	0.97±0.48	1.70±0.45	2.10±0.95
	47.28	49.60	26.36	45.08
AUC/dose	4.92±1.82	4.7±2.17	3.76±1.68	5.69±2.16
	37.05	46.09	44.67	37.97
Vd/F (L)	354.36±146.02	355.90±132.70	356.78±72.50	336.35±144.51
	41.21	37.29	20.32	42.96
CL/F (L/hr)	284.56±199.04	244.39±95.53	253.10±81.94	219.06±79.05
	69.95	39.09	32.38	36.08
$T_{1/2}$ (hr)	1.13±0.29	1.08±0.37	1.03±0.28	1.08±0.30
	25.19	34.62	27.23	27.95

Table 15 Pharmacokinetics Parameters in Male and Female Subjects after Administration of INSCOP at Two Dose Levels under Ambulatory Condition.

Parameters (Mean±SD)	Unit	Dose (0.2mg)		Dose (0.4mg)	
		female	male	female	male
N		6	6	6	6
C_{max}	(ng/mL)	0.34±0.19	0.19±0.09	1.02±0.63	1.11±0.28
Tmax	(hr)	1.21±0.49	1.38±0.43	1.17±0.20	1.39±0.48
Ka	(hr^{-1})	1.48±0.91	0.86±0.34	1.12±0.48	0.86±0.33
AUC	(hr*ng/mL)	1.11±0.46	0.70±0.31	1.76±0.24	1.66±0.59
Vd/F	(L)	360.66±146.36	348.07±159.35	334.02±64.55	375.74±78.93
CL/F	(L/hr)	202.74±71.32	366.39±256.91	231.15±30.12	271.39±108.72
T1/2	(hr)	1.24±0.26	1.03±0.29	1.01±0.26	1.04±0.32

* C_{max}, maximum plasma concentration;

T_{max}, time to reach maximum concentration;

K_a, absorption rate constant;

AUC, area under the plasma concentration-time profile curve;

Vd/F, apparent volume of distribution;

CL/F, apparent total body clearance;

T½, terminal elimination half-life;

SD, standard deviation.

Table 16. Mean Ratios of PK Parameters between AMB and ABR Conditions in 12 Subjects

PK parameters (Mean±SD)	Dose(mg)	
	0.2	0.4
	ABR/AMB	
C_{max}(ng/mL)*	1.60±1.12	1.36±0.30
C_{max}/dose*	1.19±0.47	1.56±0.45
T_{max}(hr)*	0.81±0.31	0.82±0.25
K_a(hr^{-1})*	1.81±1.40	1.71±0.89
AUC(hr*ng/mL)*	1.35±0.98	1.26±0.45
AUC/dose*	1.02±0.50	1.42±0.52
Vd*F(L)*	1.17±0.50	0.93±0.37
CL/F(L/hr)*	1.01±0.50	0.90±0.32
$T_{1/2}$(hr)*	0.94±0.20	1.12±0.50

Parameter*: The ratio of PK parameters between tow gravity condition groups.

4.4. Compartmental pharmacokinetic modeling

From the previous clinical studies, we have Twenty-four healthy subjects in ambulatory condition. We quantified not only the plasma concentration of scopolamine but also the saliva and urinary concentrations of scopolamine. There was no studies focus on the co-modeling of drug concentrations in plasma, saliva and urine compartments; therefore we were trying to establish the correlationships among these three compartments, using modeling software. We performed PK modeling combining all data of ambulatory subjects. Basically, we built a model structure for scopolamine in plasma, saliva and urine compartments, by using Phoenix software. We hypothesized that there were predictable correlations among scopolamine concentrations of in compartments of plasma, saliva and urine.

4.4.1. Scopolamine and metabolite profiles in plasma, saliva and urine

Among the twenty four healthy human subjects, twelve subjects from Aim 1 (Data Set 1) were used to perform the model fitting. The Data Set 2 from Aim 2 were used to validate the model. The plasma, saliva and urine samples of healthy human subjects were analyzed using the validated LC-MS/MS method. The plasma concentration versus time profiles of scopolamine and SCOP-G for Data Set 1 are presented in Section4.1.3. In Section 4.1.6, the saliva concentrations of scopolamine and SCOP-G from **Data Set 1** were presented and the urinary cumulative excretion amount versus time plots for scopolamine and SCOP-G are shown in **Figure 29** and **Figure 30.**

The plasma concentration versus time profiles of scopolamine and SCOP-G from Data Set 2 are presented in Section4.3.2.The saliva concentration of scopolamine and SCOP-G for **Data Set 2** are presented in **Figure 31** and **Figure 32.**

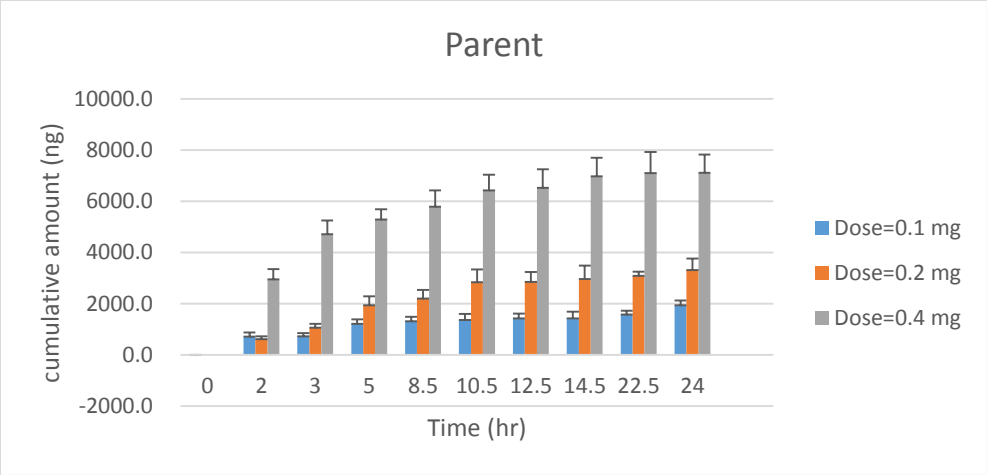


Figure 29. Mean Cumulative Excretion Amount of Scopolamine versus Time Bar Graphs of 12 Subjects (Data Set 1) at Three Dose Levels. Error bars corresponded to standard deviations.

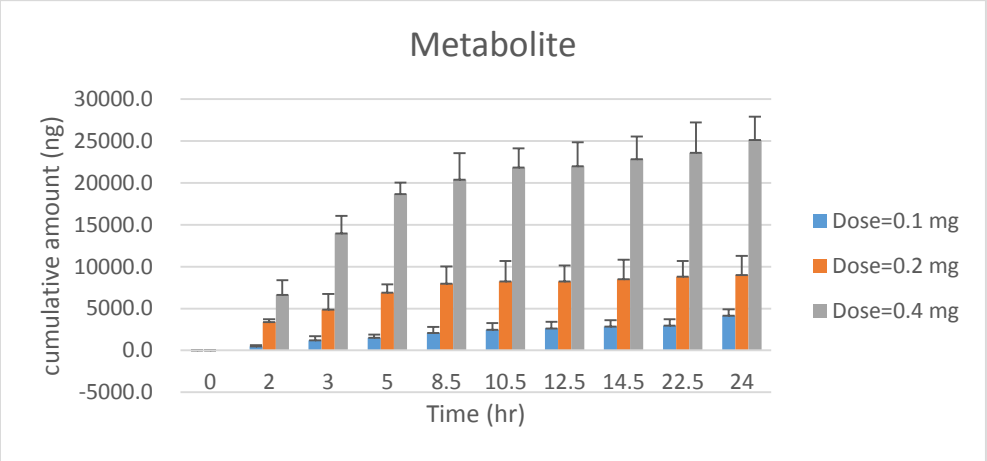


Figure 30. Mean Cumulative Excretion Amount of SCOP-G versus Time Bar Graphs of 12 Subjects (Data Set 1) at Three Dose Levels. Error bars corresponded to standard deviations. Error bars corresponded to standard deviations.

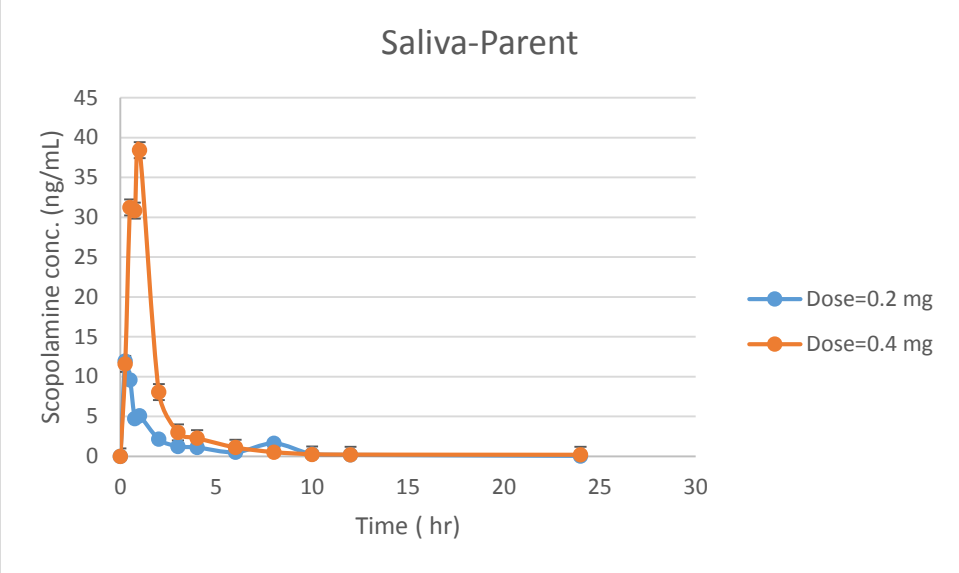


Figure 31.Mean Saliva Concentration of Scopolamine versus Time Plots of 12 Subjects (Data Set 2) at Two Dose Levels

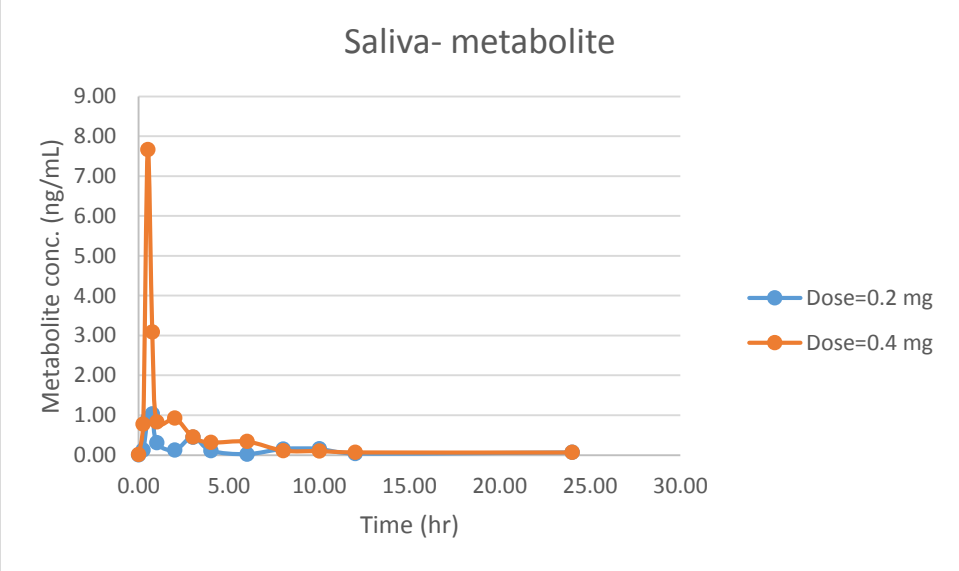


Figure 32. Mean Saliva Concentration of SCOP-G versus Time Plots of 12 Subjects (Data Set 2) at Two Dose Levels

4.4.2. Partition coefficient ratio of scopolamine between saliva and plasma

The distribution mechanism of compounds between plasma and saliva compartments were evaluated by calculated the partition coefficient ratio of saliva and plasma (Ks/p). The AUC ratio between saliva and plasma versus time profiles were derived. The equilibrium K values were determined for scopolamine and SCOP-G at different dose levels.

The Ks/p versus time profiles of scopolamine at 0.1, 0.2 and 0.4 mg doses are presented in Figures 33, 34 and 35, respectively. The Ks/p profiles of SCOP-G were presented in Figure 36, 37 and 38, respectively.

The equilibrium Ks/p between saliva and plasma are 47, 28 and 29 for scopolamine when the values were 7.2, 4.3 and 4.1 for SCOP-G at 0.1, 0.2 and 0.4 mg doses, respectively.

After statistical analysis, the time for SCOP to reach the equilibrium K were 2, 1 and 1 hour; while the time for SCOP-G were 2, 2 and 2 hours after administration of INSCOP at 0.1, 0.2 and 0.4 mg doses, respectively.

The partition coefficient ratios between saliva and plasma (Ks/p) of scopolamine were 5~6 times higher than those of the SCOP-G at 0.1, 0.2 and 0.4 mg doses, respectively. The Ks/p after 0.1 mg dose was higher than that at 0.2 and 0.4 mg doses indicating a significant salivary distribution of SCOP and SCOP-G at the low dose.

These results suggested that there was a non-linear PK involved in transfer of SCOP and SCOP-G between plasma and saliva compartments.

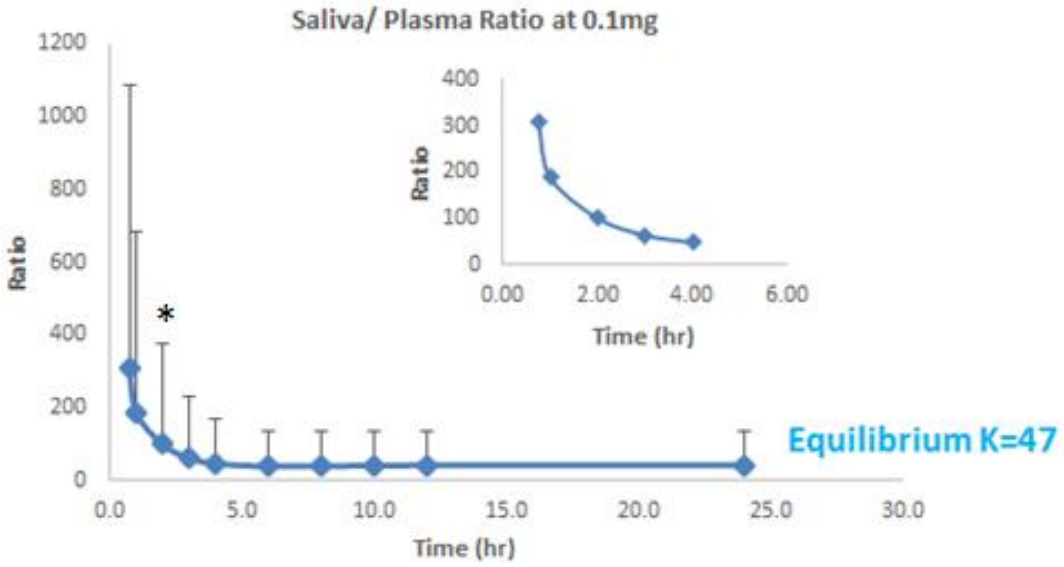


Figure 33 Partition Coefficient Ratios of Scop between Saliva and Plasma (Saliva/Plasma) versus Time Profile at 0.1 mg Dose

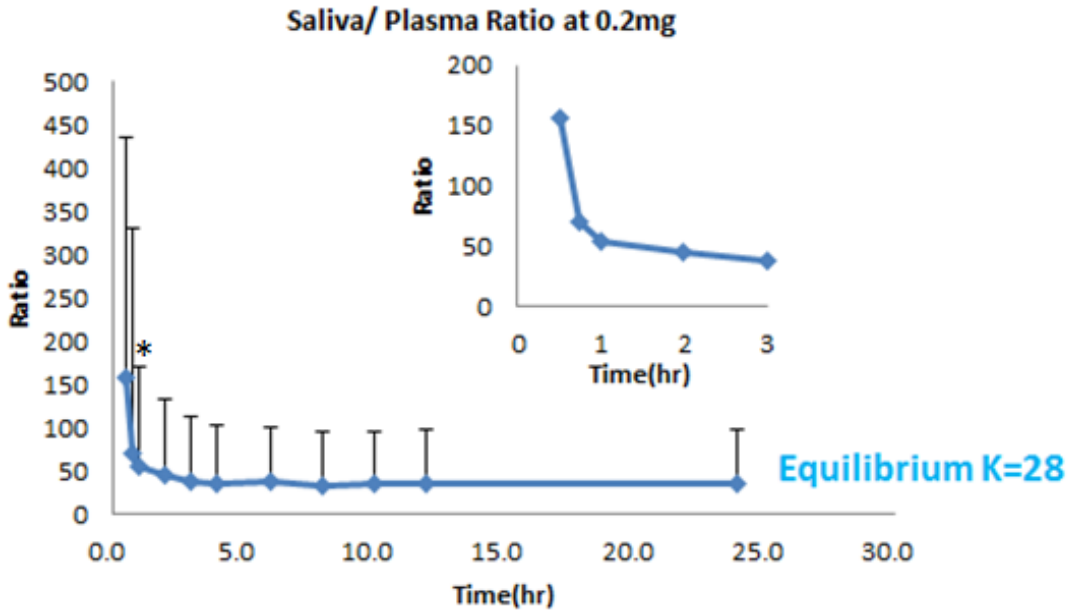


Figure 34 Partition Coefficient Ratio of Scop between Saliva and Plasma (Saliva/Plasma) versus Time Profile at 0.2 mg Dose

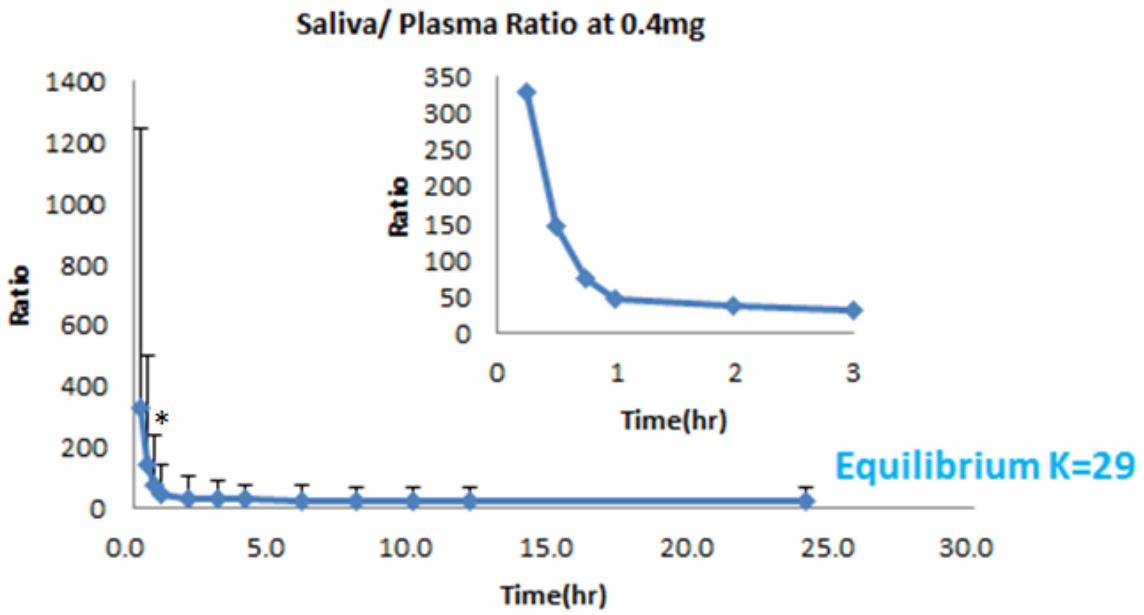


Figure 35 Partition Coefficient Ratio of Scop between Saliva and Plasma (Saliva/Plasma) versus Time Profile at 0.4 mg Dose

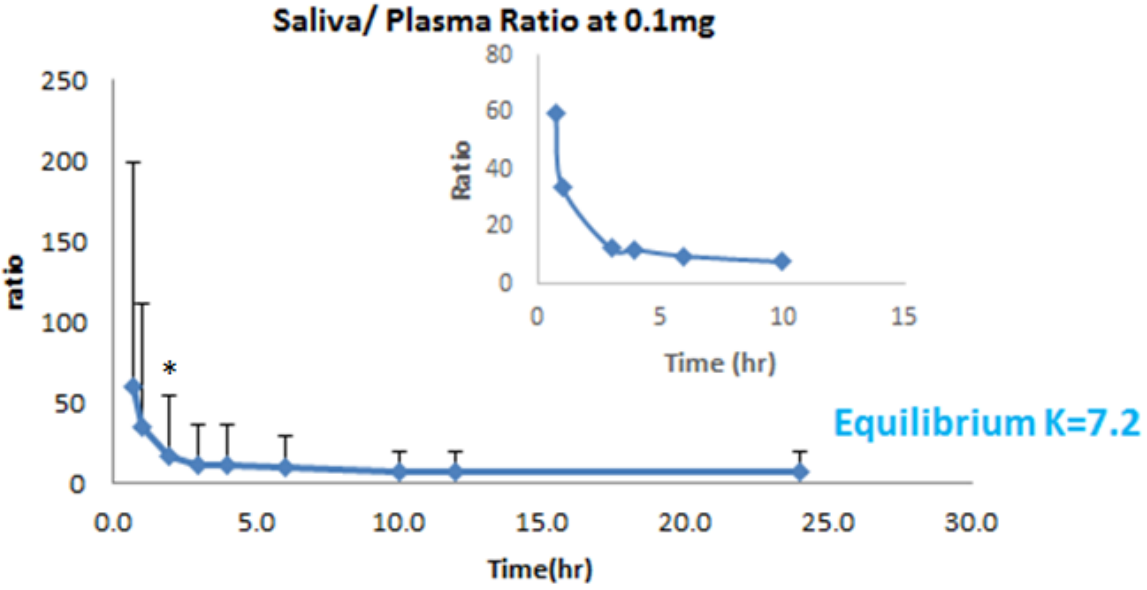


Figure 36 Partition Coefficient Ratio of Scop-G between Saliva and Plasma (Saliva/Plasma) versus Time Profile for SCOP-G at 0.1 mg Dose

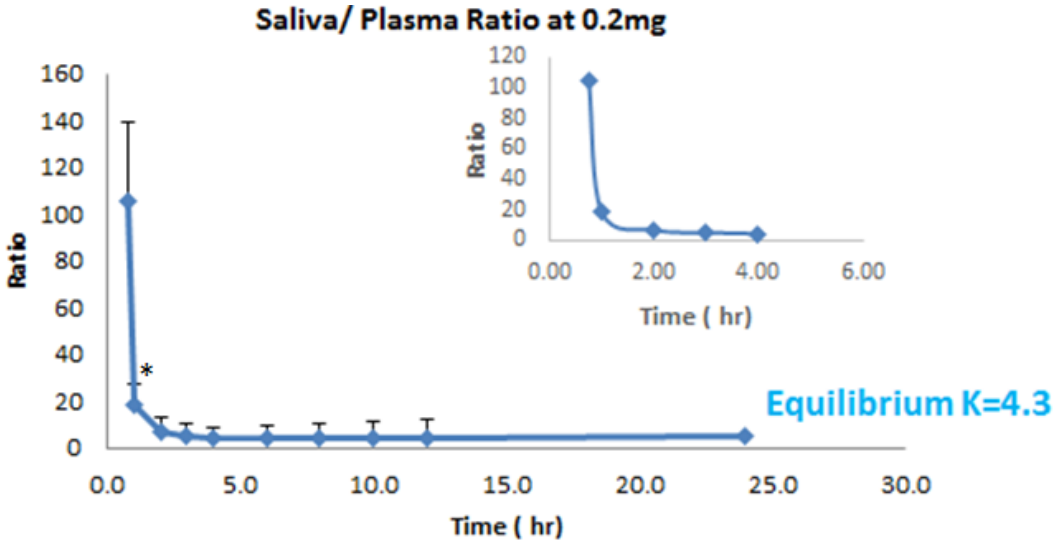


Figure 37 Partition Coefficient Ratio of Scop-G between Saliva and Plasma (Saliva/Plasma) versus Time Profile for SCOP-G at 0.2 mg Dose

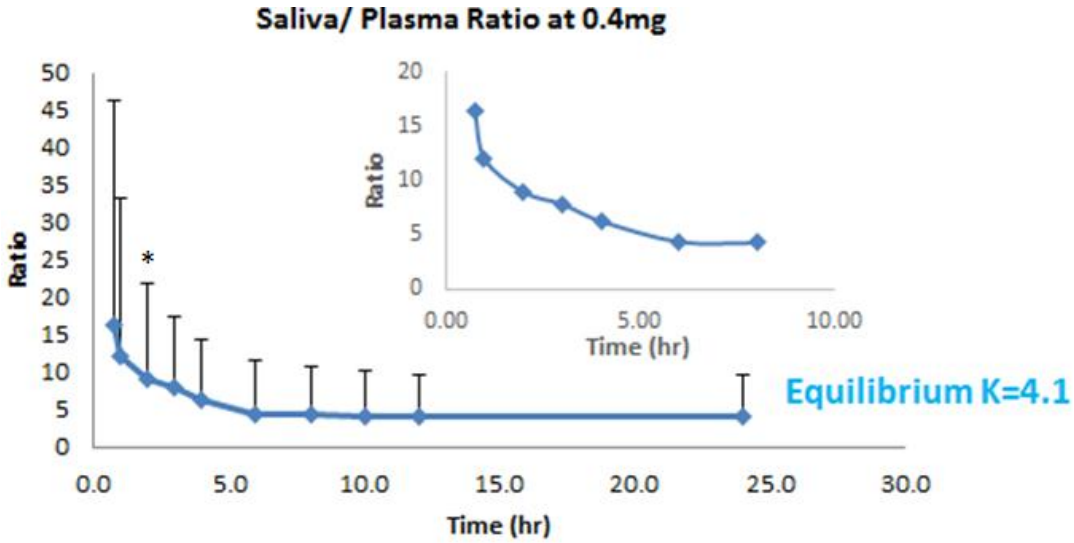


Figure 38 Partition Coefficient Ratio of Saliva/Plasma versus Time Profile for SCOP-G at 0.4 mg Dose

4.4.3. Optimization of pharmacokinetic models

We constructed several pharmacokinetic (PK) models based on the SCOP PK concentrations in plasma, saliva and urine from 12 healthy subjects (Data Set 1) and compared the goodness of fit results among different models. With the model optimization, we developed and found the best fit model to describe the pharmacokinetics of SCOP in plasma, saliva and urine.

4.4.3.1 PK model structure for scopolamine and SCOP-G.

Base on the physiological and pharmacological factors, we built Model A with six compartments with observed drug concentration of scopolamine in plasma, saliva, and urine, respectively, as well as SCOP-G concentrations in plasma, saliva, and urine. The model structure of Model A is presented in **Figure 39**. The model was described by the mass balance equations listed below:

dx1/dt = -Ka*C1

dx2/dt = Ka *C1+K42 *C4-(K24+K25*+Km)*C2

dx3/dt = Km*C2 + K43*C4 – (K34+K35*+Ke,m)*C3

dx4/dt = K24 *C2 + K34 * C3 – (K42+ K43)*C4

dx5/dt = K25*C2 + K35* C3

The best fit profiles of SCOP and SCOP-G in plasma, saliva and urine are presented in **Figure 40.**

From the best fit profiles we concluded that urinary concentration profiles of SCOP and SCOP-G were adequately described by Model A , while no good correlations were established between observed concentrations and predicted concentrations in plasma and saliva compartments in Model A

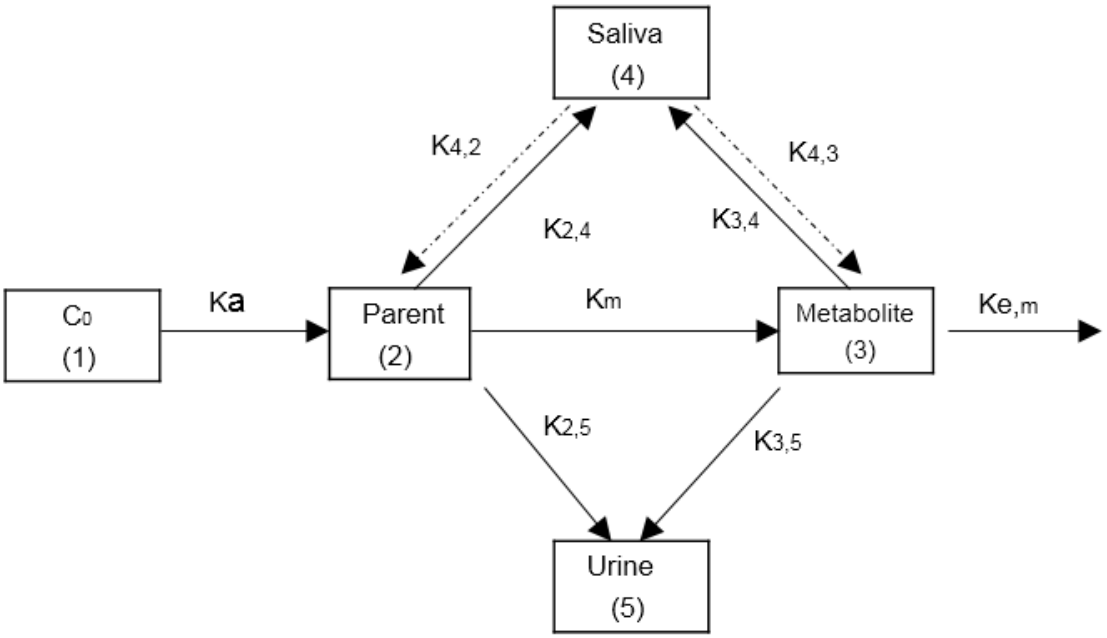


Figure 39. The Model Structure of Scopolamine and SCOP-G with Model A

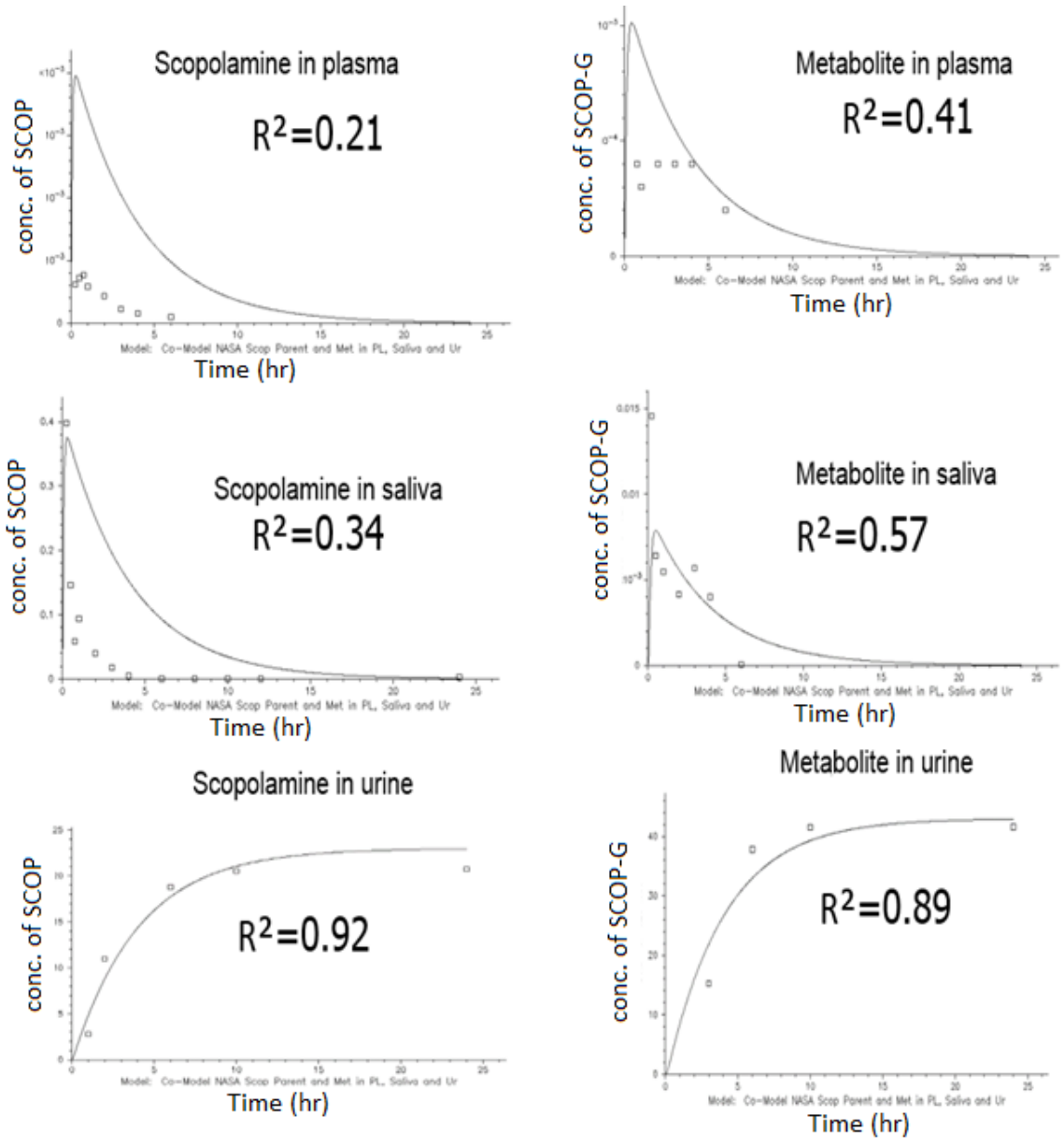


Figure 40. Best Fit Profiles of Scopolamine and SCOP-G in Plasma, Saliva and Urine with Model A

4.4.3.2 PK model structure for scopolamine

The result from Section 4.4.3.1 indicated that model A was over parameterized resulted with poor parameter precision and poor correlation of observed and predicted data. With the model structure B of SCOP in plasma, saliva and urine, the metabolisms in plasma and saliva were collectively included in the model, by PK parameters of Kpout and Ksout.

The structure of Model B is presented in **Figure 41**. The model was described by the mass balance equations listed below:

dx1/dt = -Ka*C1

dx2/dt = Ka *C1+Ksp *C4-(Kps+Kpu+Kpout) *C2

dx3/dt = Kpu*C2

dx4/dt = Kps *C2 – (Ksp +Ksout) * C4

The best fit profiles of SCOP in plasma, saliva and urine are presented in **Figure 42**. From the best fit profiles, we concluded that significant data fitting in plasma and saliva compartments were improved with R^2 increasing from 0.21 to 0.91 for plasma profile and 0.34 to 0.78 for saliva profile. While A good correlation between observed and predicted concentration in urine were maintained (R^2=0.92 in Model A and 0.98 in Model B). An over-estimation were shown in saliva compartment, and the model needed to be optimized.

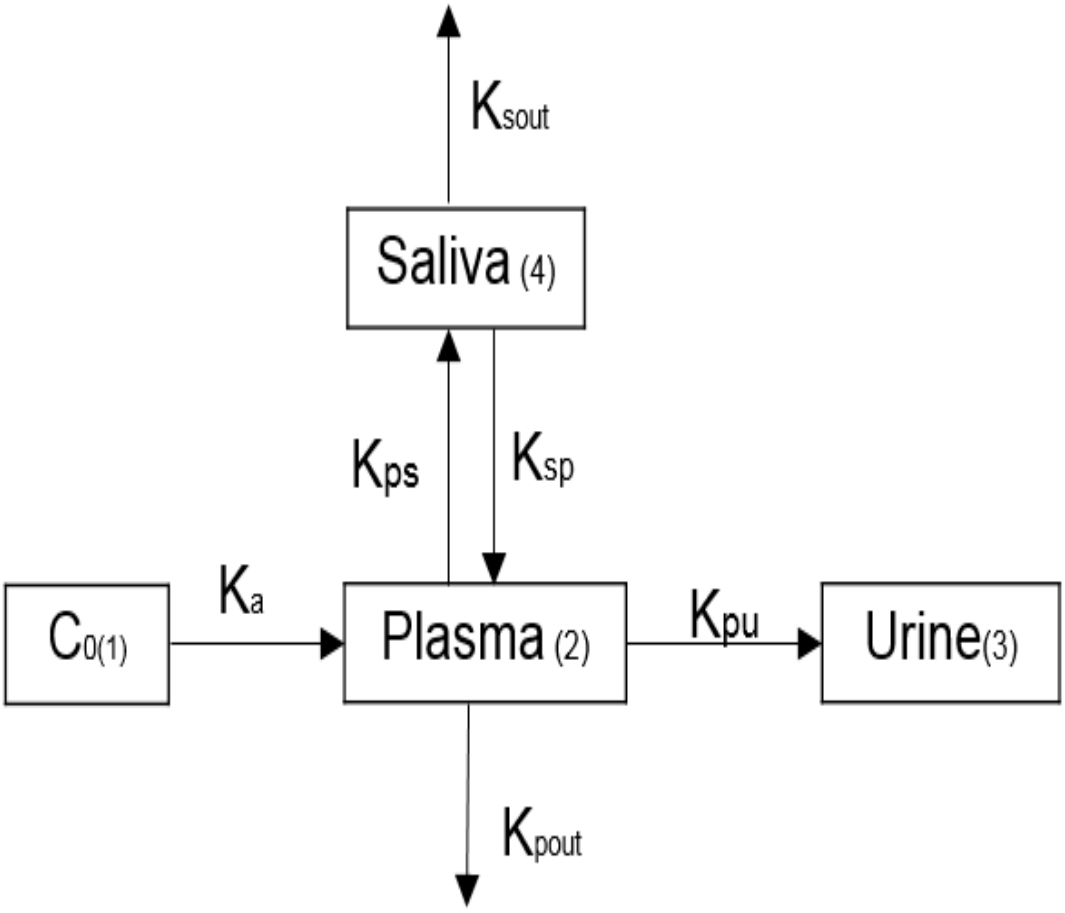


Figure 41. Model Structure of SCOP with Model B

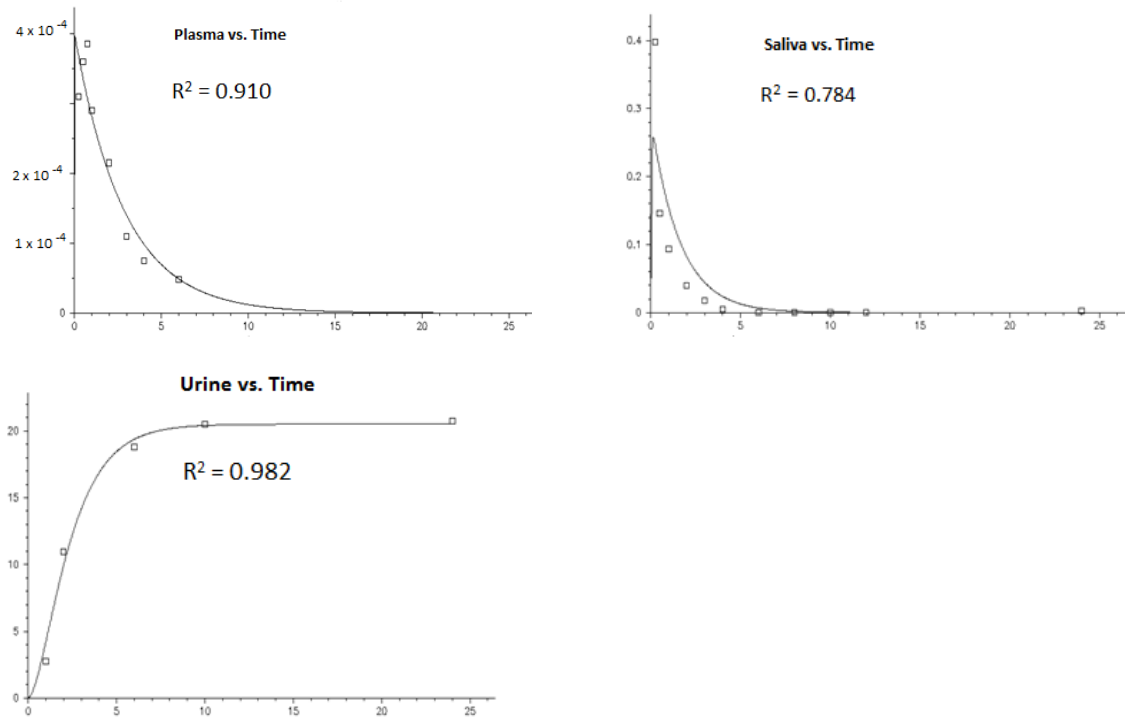


Figure 42. Best Fit Profiles of SCOP in Plasma, Saliva and Urine with Model B

4.4.3.3 PK model structure for scopolamine with non-linear PK

From the results of partition coefficient ratio of saliva/plasma study, we concluded that there was a saturation of SCOP distribution between plasma and saliva compartments. The non-linera PK parameters Vmax and Km were added into the Model B, and K_{a2} was added to saliva compartment. The final structure of Model C are presented in **Figure 43**. The model was described by the mass balance equations listed below:

dx1/dt = -(Ka1+Ka2)*C1

dx2/dt = Ka1 *C1+Ksp *C4-{Vmax/(Km+C2)}*C2-(Kpu+Kpout) *C2

dx3/dt = Kpu*C2

dx4/dt = {Vmax/(Km+C2)} *C2 +Ka2 *C1-(Ksp + Ksout) * C4

The best fit profiles of scopolamine in plasma, saliva and urine are presented in **Figure 44**.

The results indicated that the inclusion of non-linear PK transferring from plasma to saliva compartments resulted in a significant improvement in the model fitting. The scopolamine concentrations in plasma, saliva and urine could be best described by the final model C with R^2 of 0.926, 0.944 and 0.979 for SCOP profiles in plasma, saliva and urine, respectively.

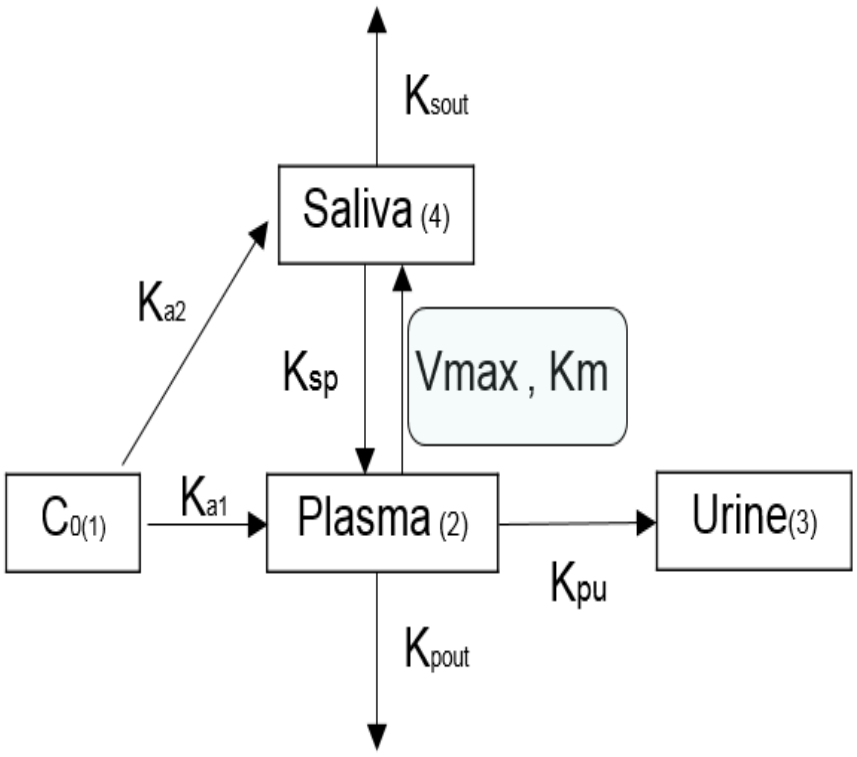
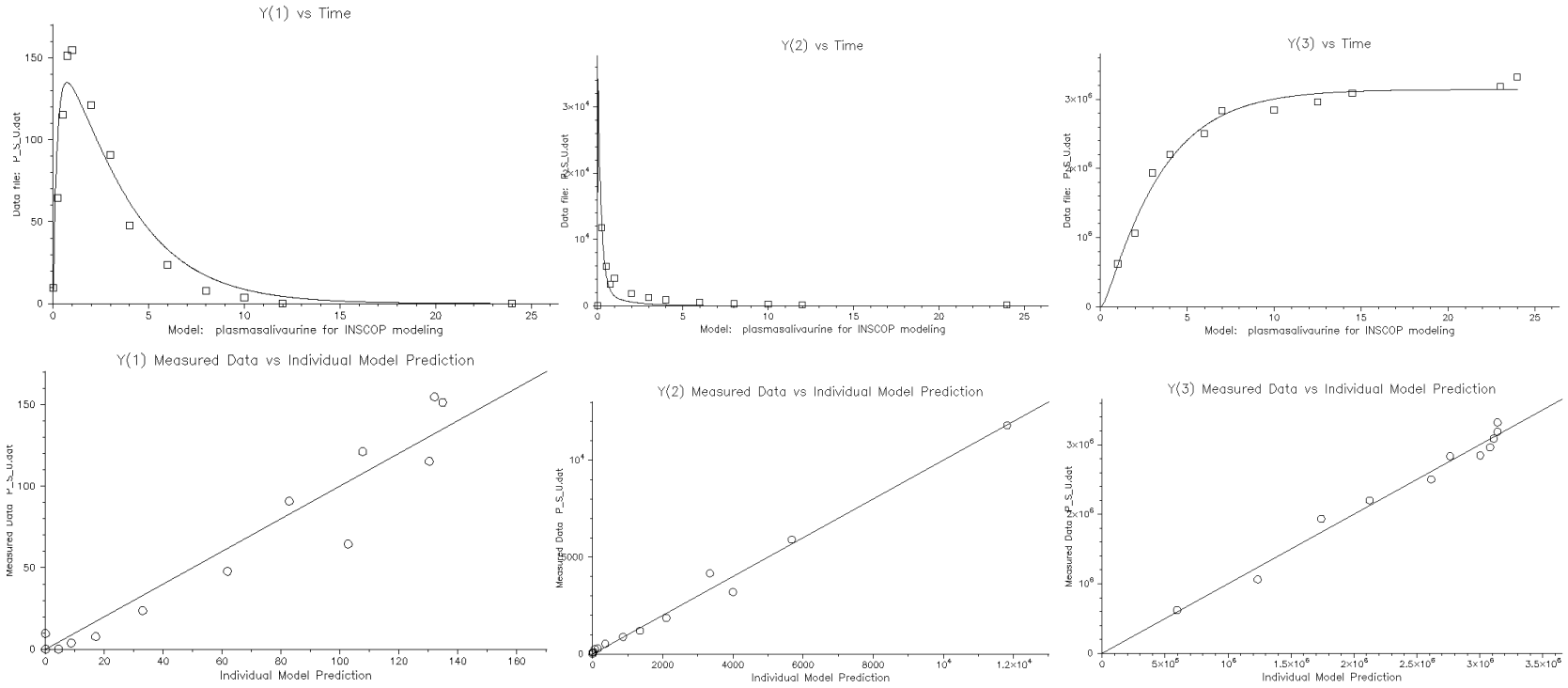


Figure 43. Model Structure of SCOP with Model C

Plasma vs. Time	Saliva vs. Time	Urine vs. Time



$R^2 \simeq 0.926$	$R^2 \simeq 0.944$	$R^2 \simeq 0.979$

Figure 44. Best Fit Profiles of SCOP in Plasma, Saliva and Urine with Model C

4.4.4. Individual Compartmental pharmacokinetic model analysis

Plasma concentration, saliva concentration and urine data collected over 24 h were used for the development of PK models. The results obtained using different models tested in this study were summarized in **Table 17**. The best fit structural model for SCOP (minimal AIC =907.2) consisted of compartments for plasma, saliva and urine, connected with different rate constants. The model fitting exercises revealed nonlinear PK process of SCOP transferring from plasma to saliva compartment with V_{max} and K_m. The best fit model structure (Model C) was shown in **Figure 45.** Individual analysis from the best fit PK Model C were performed, the best fit profiles in plasma, saliva and urine,respectively, in each subjects at three dose levels were shown in **Figure 46.** Selected individual best fit profiles in plasma, saliva and urine are shown in **Figure 47**.Only subject01, 02, 03, 07, 08 and 09 were presented since the similarity of profiles were observed for rest of subjects. The Log sale of goodness fit plots of SCOP in plasma, saliva and urine, respectively, were shown in **Figure 48**. Good correlations were observed between observed and predicted values of SCOP in all three different compartments of plasma, saliva and urine. The PK parameters described in Model C were derived for each subjects. The mean values of each PK parameters individually predicted from Model C were summarized in **Table 18.** From the PK parameter estimations, each predicted PK parameter was comparable among the three dose levels. The volume distribution in plasma were 597 (43.2%), 821 (64.7%) and 639 (57.1%) L for 01, 0.2 and 0.4 mg dose, respectively. The volume distribution in saliva are 41.5 (87.2%), 37.4 (56.1%) and 29.3 (35.4%) for 01, 0.2 and 0.4 mg dose, respectively.

The diagnostic plots for model discrimination are shown in **Figure 49** and **Figure 50**. The Figure 49 represented the weighted residuals versus time Profile, in which ,all

weighted residuals fall within the narrow range of -2 to 2, and distributed around the line of 0 on Y axis in all the three compartments. Weighted residuals versus standard normal quantiles profiles in the three compartments are presented in **Figure 50**. All weighted residuals followed the line of identity, which indicated that there was a normal distribution of residuals. The diagnostic plots from PK modeling indicated that the final best fit model of SCOP from INSCOP could simultaneously describe the plasma, saliva and urinary data for all subjects at the three dose levels with reliability and stability.

Table 17. Model Build-Up Summary for Scopolamine

Model No.	Model description	AIC
1p (Model A)	Plasma, saliva and urine(SCOP and SCOP-G) 1 compartment of plasma; Kps and Ksp for saliva	10275
2p	Plasma, saliva and urine (SCOP) 1 compartment of plasma; Kps and Ksp for saliva	1024.5
3p	Plasma, saliva and urine (SCOP) 1 compartment , Kpout for plasma; Kps and Ksp for saliva	1022.7
4p(Model B)	Plasma, saliva and urine (SCOP) 1 compartment, Kpout for plasma; Kps,Ksp and Ksout for saliva	976
5p*(Model C)	Plasma, saliva and urine (SCOP) 1 compartment, Kpout for plasma Ka, Vmax, Km,Ksp and Ksout for saliva	902.7

* Model 5p is the best fit structural model for Scopolamine, consisting of plasma, saliva and urine compartments, with non-linear distribution from plasma to saliva.

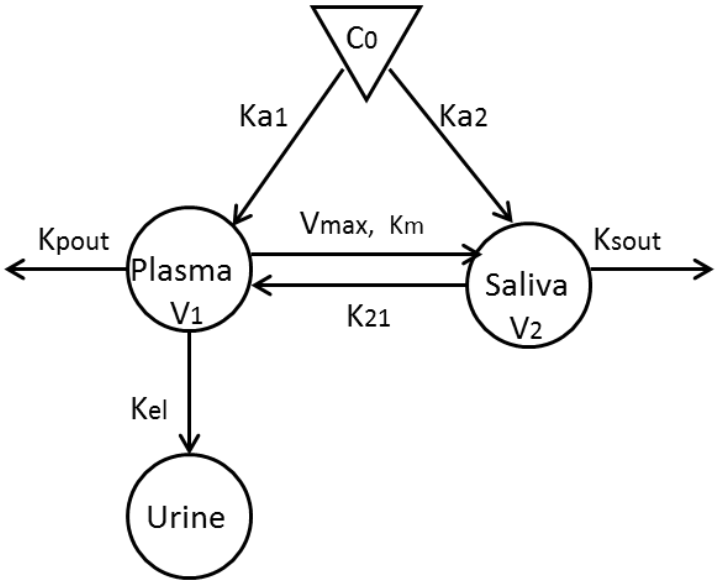


Figure 45. Best Fit Model Structure of SCOP with Nonlinear PK Distribution from Plasma to Saliva.

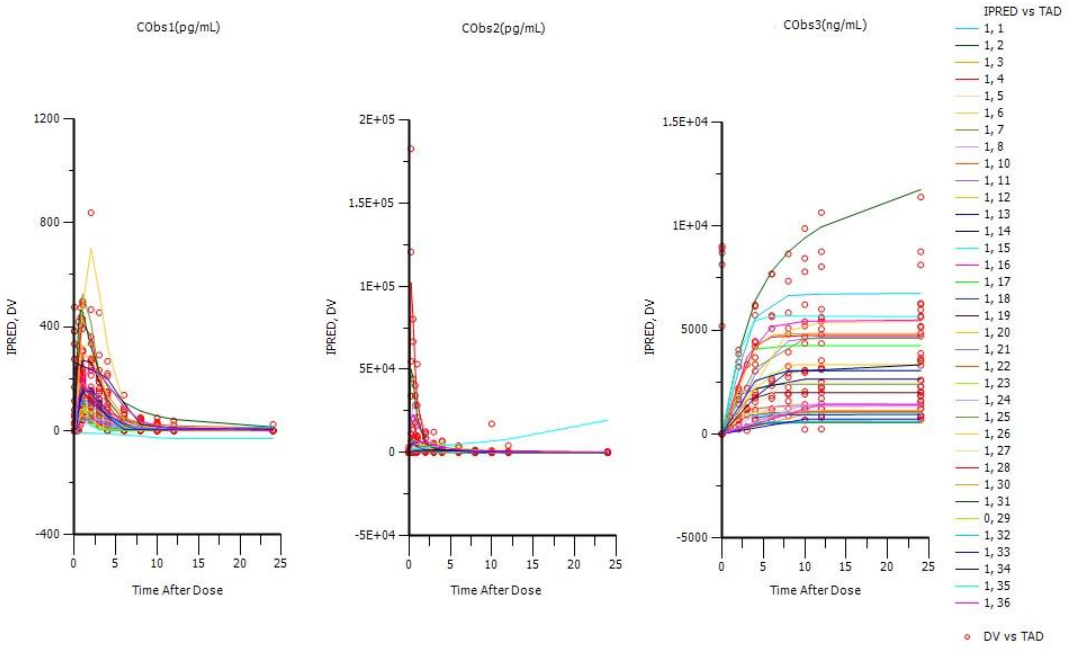


Figure 46. Best Fit Profiles of 12 Subjects at Three Dose Levels (Data Set 1)

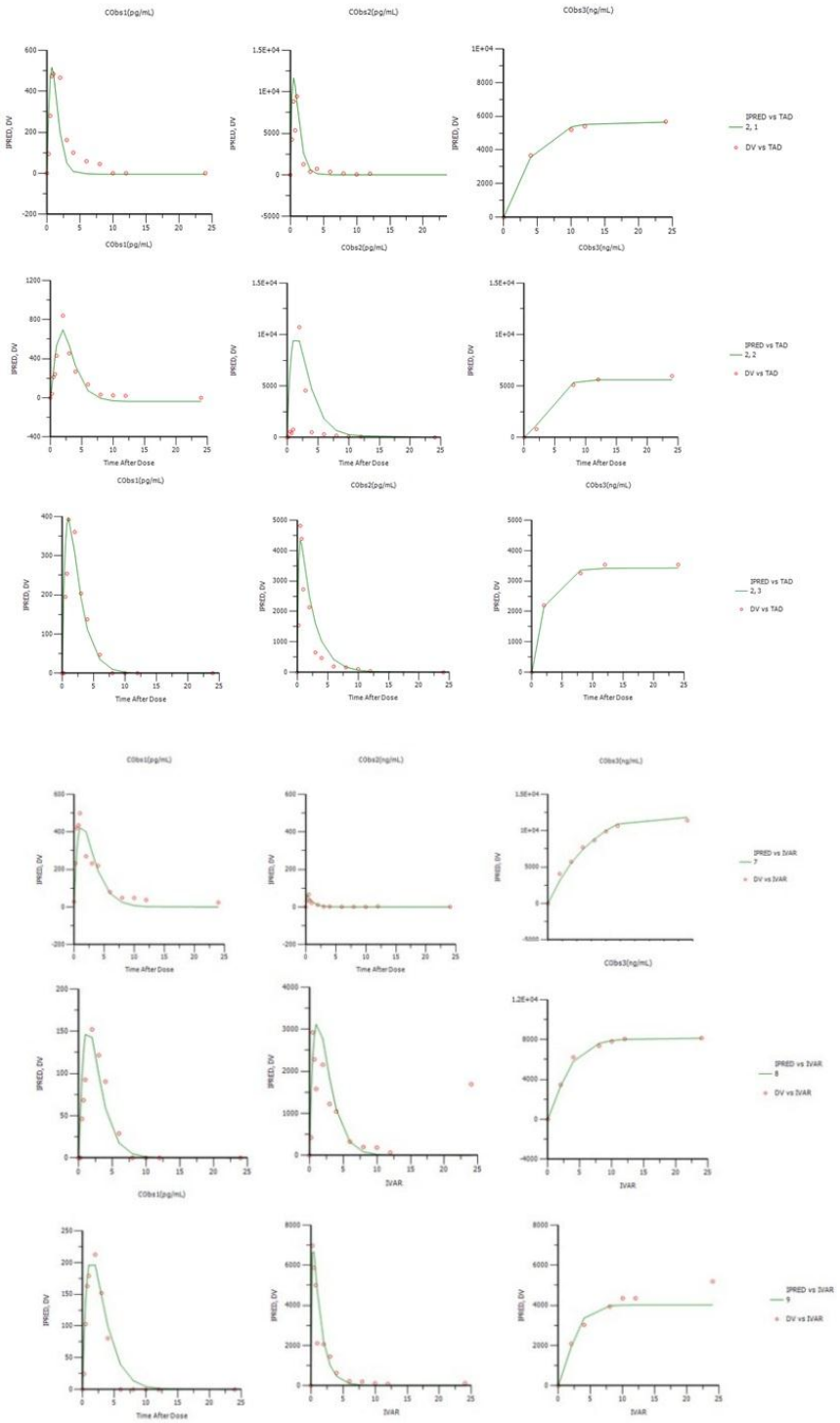


Figure 47. Best Fit Profiles of Selected Individuals (Subject 01, 02, 03, 07, 08, and 09) in Plasma Saliva and Urine

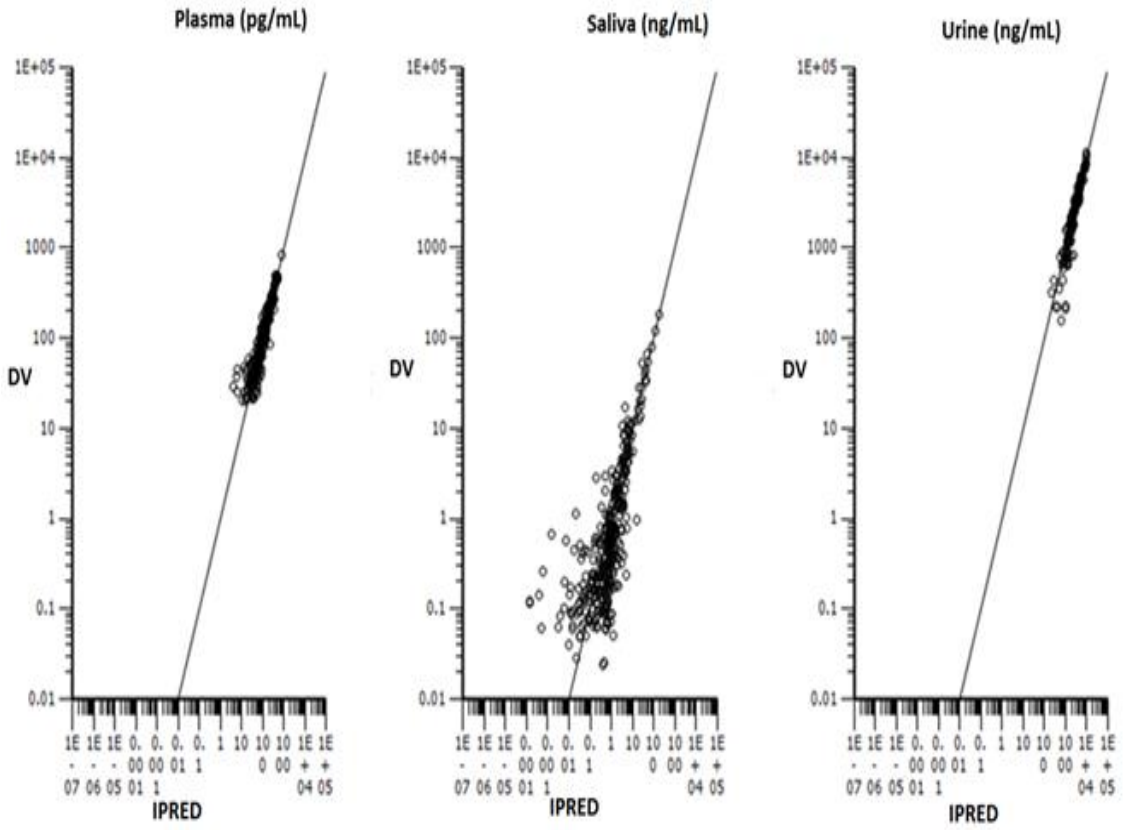


Figure 48. Goodness of Fit Plots of PK Model C (DV – Observed Concentration, IPRED-Individual Predicted Concentration) for 12 Subjects at Three Doses.

Table 18. PK Parameters Individually Predicted from PK Model C at Three Dose Levels

Parameter	Unit	Dose (mg)					
		0.1		0.2		0.4	
		Mean	CV%	Mean	CV%	Mean	CV%
K_{a1}	1/hr	0.34	74.3	0.42	69.3	0.37	47.2
K_{a2}	1/hr	0.72	68.3	0.81	63.2	0.93	57.5
K_{pout}	1/hr	0.59	43.6	0.69	38.4	0.61	29.7
K_{el}	1/hr	0.01	24.3	0.01	28.3	0.01	21.6
K_{sout}	1/hr	0.17	39.8	0.1	40.9	0.12	34.1
V_{max}	ng/hr	1698	118.6	1579	89.2	1701	77.8
K_m	pg/mL	137.2	71.9	127.7	47.5	158.3	58.4
K_{21}	1/hr	1.57	97.5	1.5	89.5	1.1	84.9
V_1	L	597	43.2	821	64.7	639	57.1
V_2	L	41.5	87.2	37.4	56.1	29.3	35.4

* K_{a1}: plasma compartment, absorption rate constant
K_{a2}: saliva compartment, absorption rate constant
K_{pout}: plasma compartment, elimination rate constant
K_{sout}: saliva compartment, elimination rate constant
K_{el}: plasma to urine rate constant
V_{max}: plasma to saliva, maximum rate
K_m: plasma to saliva, the Michaelis Menten constant
K_{21}: saliva to plasma rate constant
V_1: volume distribution in Plasma
V_2: volume distribution in saliva

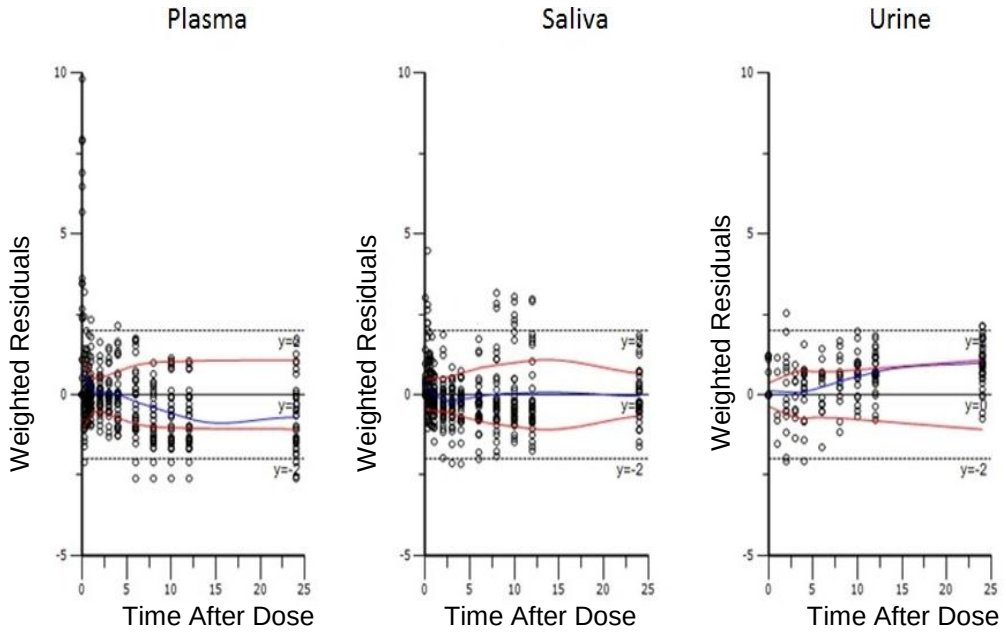


Figure 49. Weighted Residuals versus Time Profiles of 12 Subjects in Plasma, Saliva and Urine at Three Doses

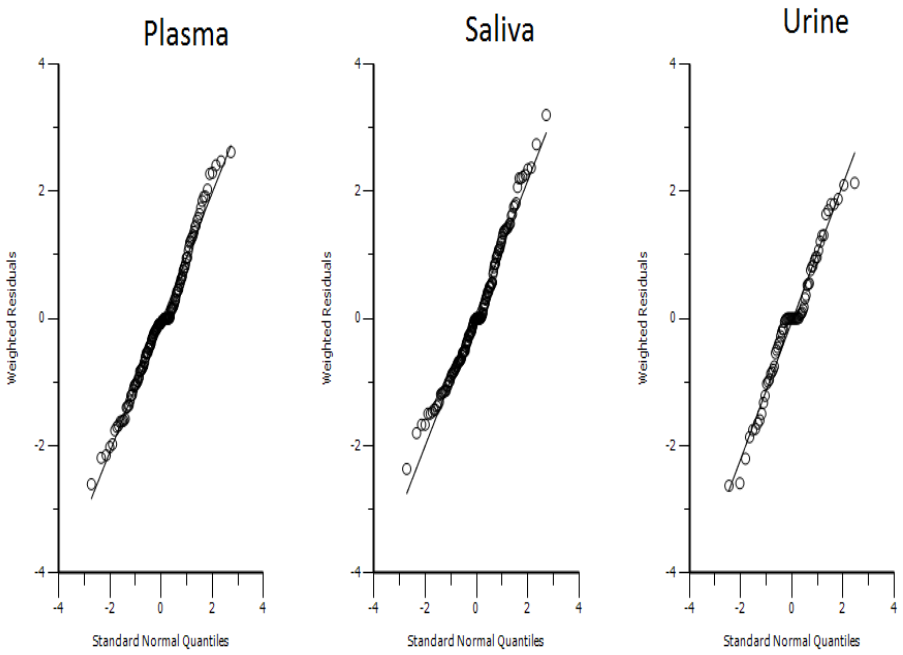


Figure 50. Weighted Residuals versus Standard Normal Quantiles Profiles 12 Subjects in Plasma, Saliva and Urine at Three Doses

4.4.5. Model validation

The identified final best fit PK model (Model C) was validated by internal and external validation methods.

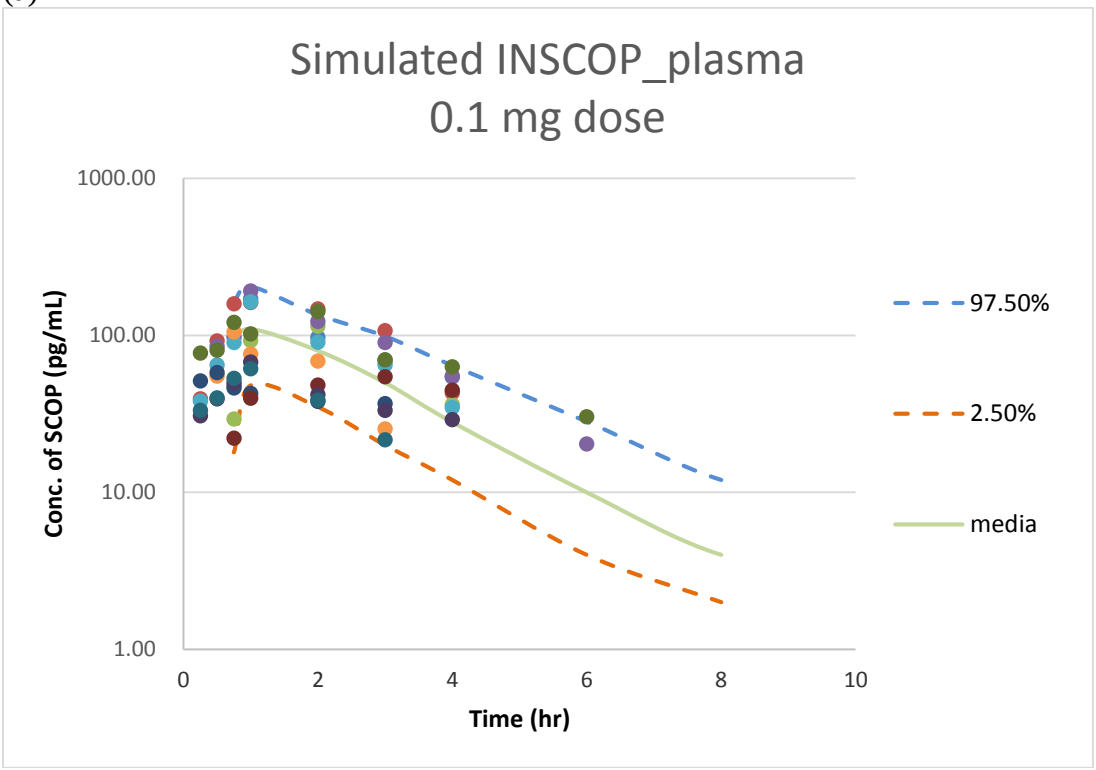
From the original data set (Data Set 1), which were used to perform the data fitting and model building, 500 data sets were successfully generated. Final estimates of PK parameters (**Table 18**) were used as fixed values in model, and 2.5th, 97.5th percentiles of model estimation were obtained in plasma and saliva at three dose levels in **Figure 51** and **Figure 52**, respectively. In these two figures, the 50th percentiles, as well as 2.5th and 97.5th percentiles of the model based predictions for plasma and saliva concentration are presented together with the observed values. Most of the observed values in plasma and saliva were within the 95% CI (confidence interval) from 500 runs of simulation.

From the external Data Set 2, we plotted the observed concentration in plasma and saliva from 12 human subjects into the 95% CI of simulation profiles generated from Data Set 1. A visual prediction check (VPC) in plasma and saliva, respectively, was performed to evaluate the model stability and reliability, which are presented in **Figure 53** and **Figure 54**, respectively. From the VPC results, most of observed values in plasma fell within the 95% CI range, except some high concentrations at each time point before 2 h (**Figure 53**). The saliva concentration observed in Data Set 2 were mostly within the 95%CI only except 1 concentration at 8 h post dose with 0.2 mg dose and at 0.4 md dose by 1 h post dose (**Figure 54**).

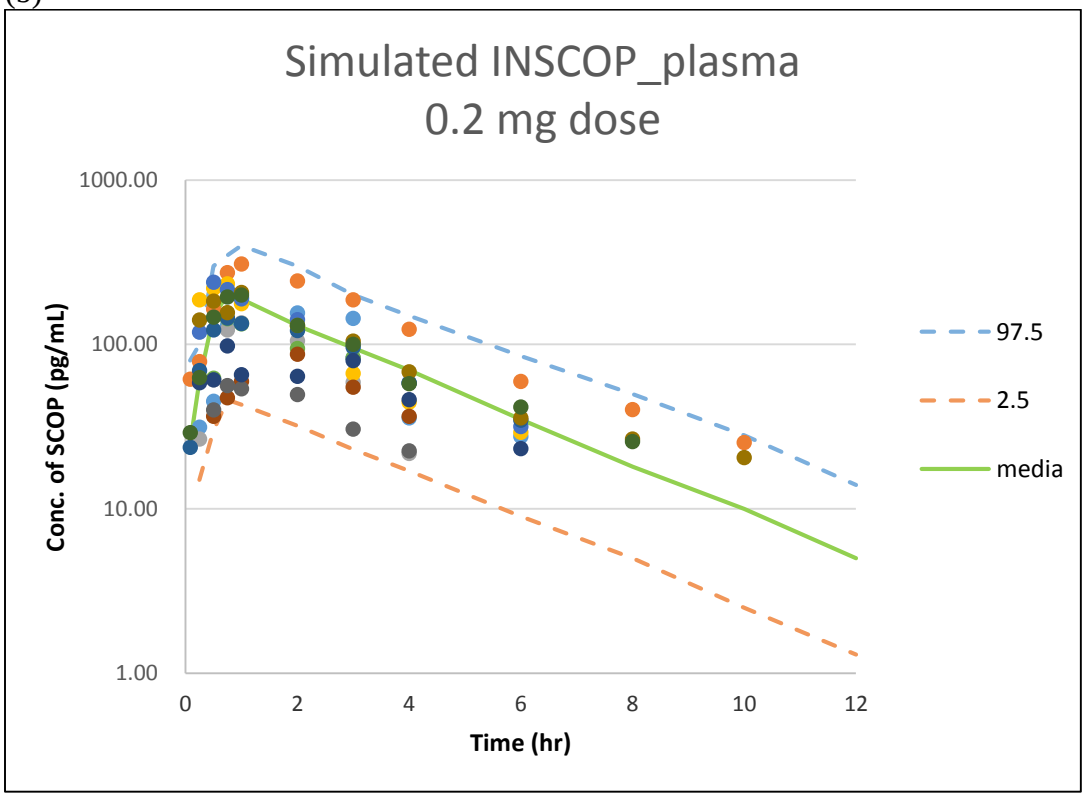
Numeral check was performed by calculating the percentage of differences between simulated and observed concentrations based on Data Set 2. **Table 19 (a) and (b)** represent the summaries of comparisons at 0.2 and 0.4 mg dose levels, respectively.

The percentage of difference between simulated and observed concentration were less than 40% before 6 hours post dose of 0.2 mg and up to 12 hours post 0.4 mg dose. The concentrations between observed and simulated values were comparable in whole concentration-time profiles at the two doses.

(a)


Simulated INSCOP_plasma
0.1 mg dose
1000.00
100.00
10.00
1.00
Conc. of SCOP (pg/mL)
0
2
4
6
8
10
Time (hr)
97.50%
2.50%
media

(b)


Simulated INSCOP_plasma
0.2 mg dose
1000.00
100.00
10.00
1.00
Conc. of SCOP (pg/mL)
0
2
4
6
8
10
12
Time (hr)
97.5
2.5
media

(c)

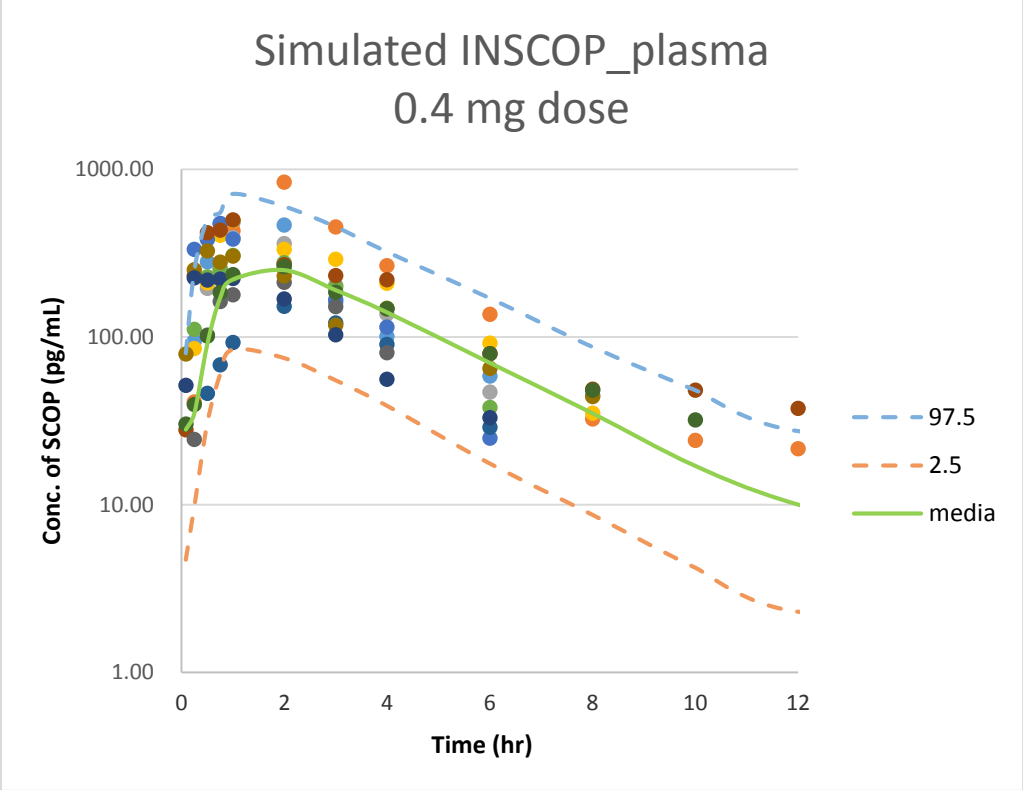
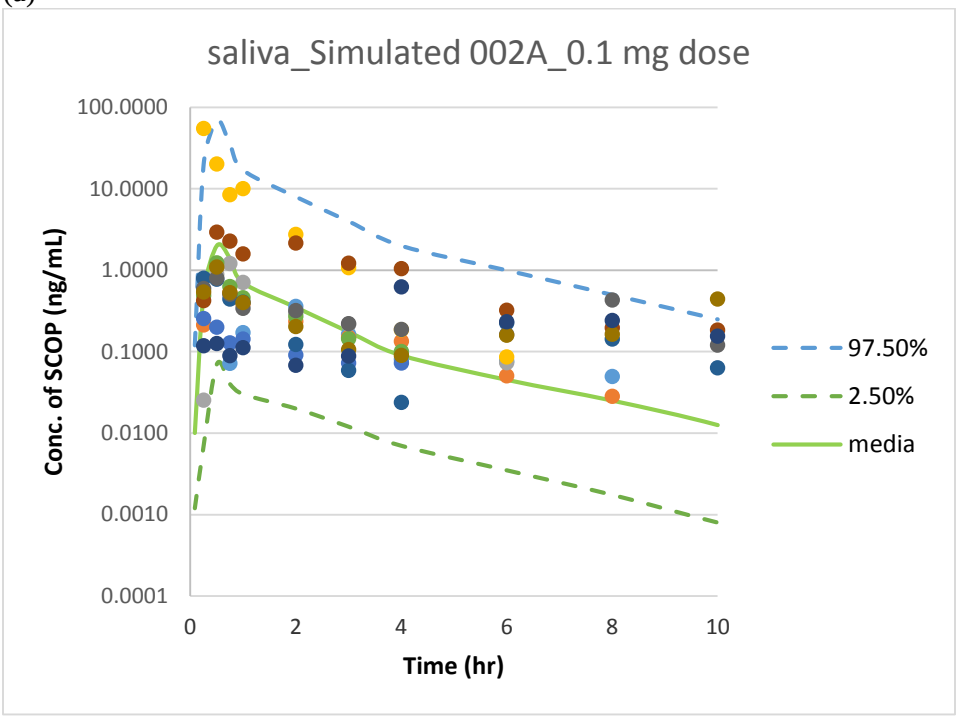
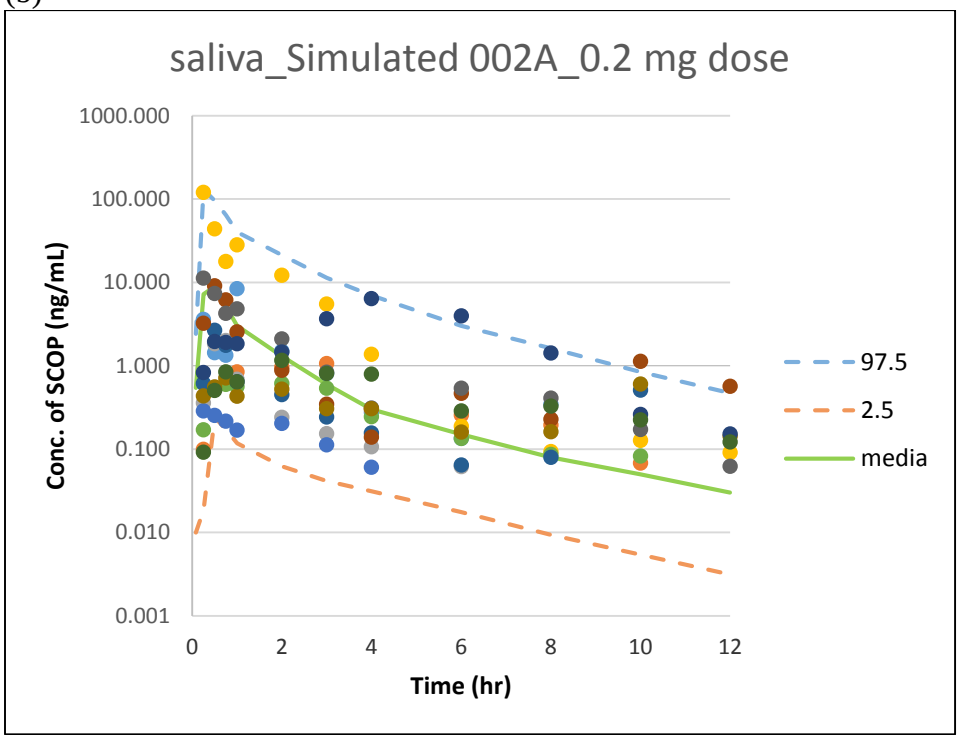


Figure 51. Simulated Plasma Concentration versus Time Profiles after 500 Simulations Post (a) 01.mg, (b) 0.2 mg and (c) 0.4 mg Doses of INSCOP Administration. Each datum point represented observed concnetration from 12 subjects. The solid line indicated 50th percentiles, and broken lines represented 2.5th and 97.5th percentiles from model estimations of 500 simulations.

(a)


saliva_Simulated 002A_0.1 mg dose
100.0000
10.0000
1.0000
0.1000
0.0100
0.0010
0.0001
Conc. of SCOP (ng/mL)
0
2
4
6
8
10
Time (hr)
97.50%
2.50%
media

(b)


saliva_Simulated 002A_0.2 mg dose
1000.000
100.000
10.000
1.000
0.100
0.010
0.001
Conc. of SCOP (ng/mL)
0
2
4
6
8
10
12
Time (hr)
97.5
2.5
media

(c)

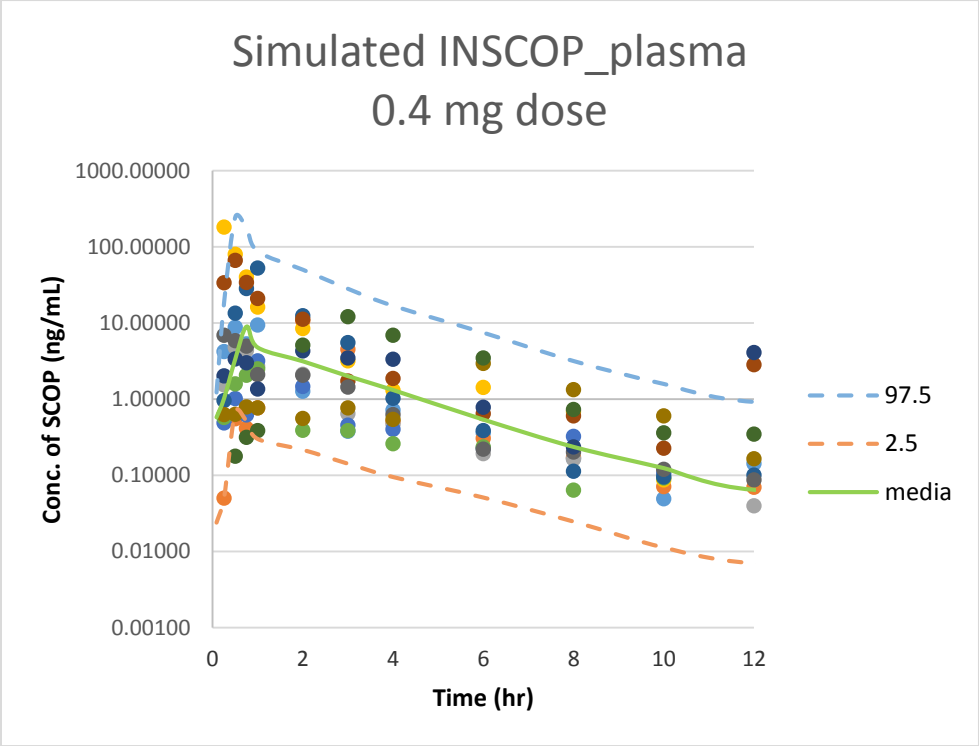
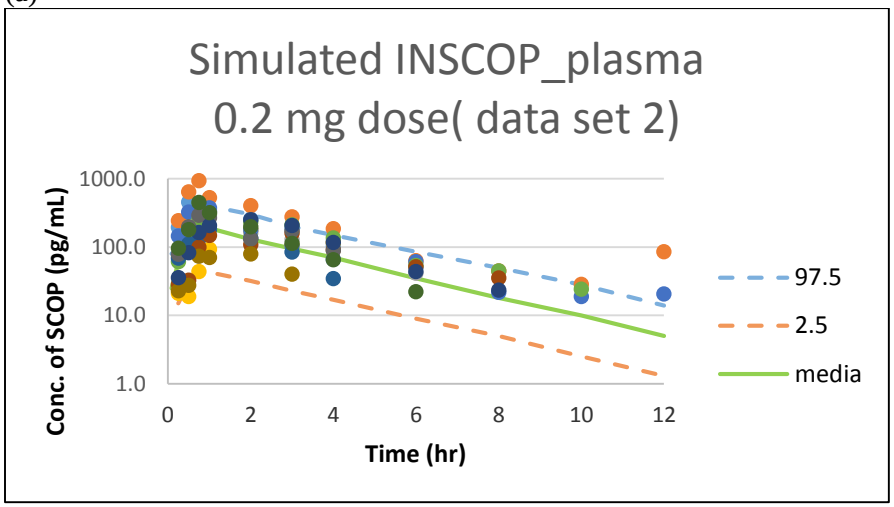


Figure 52. Simulated Saliva Concentration versus Time Profiles after 500 Simulations POST (a) 01.mg, (b) 0.2 mg and (c) 0.4 mg Dose of INSCOP Administration. Each datum point represented observed concentration from 12 subjects. The solid line indicated 50th percentiles, and broken lines represented 2.5th and 97.5th percentiles from model estimations of 500 simulations.

(a)



(b)

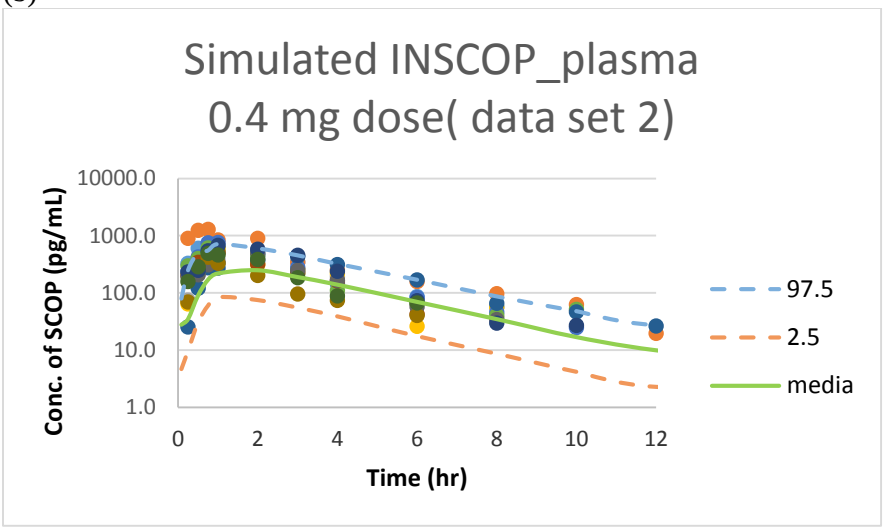
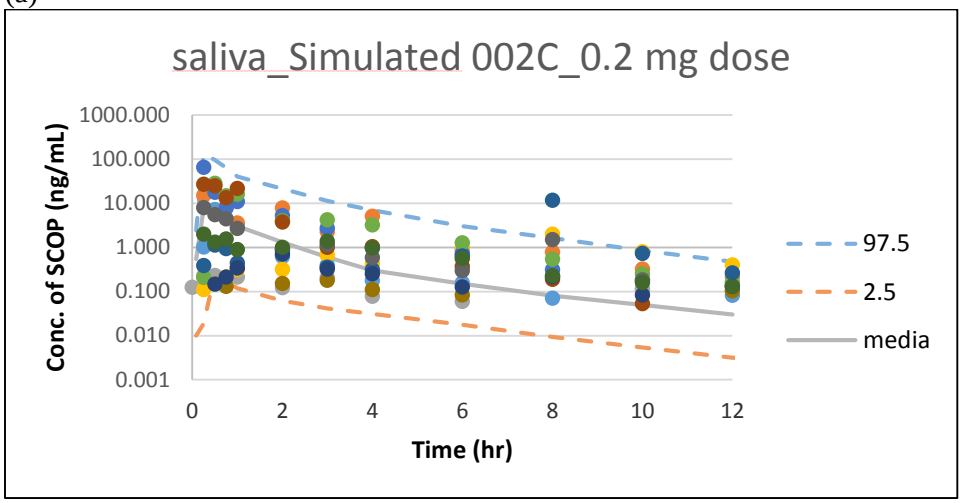


Figure 53. Visual Prediction Check (VPC) of Plasma Concentration versus Time Profiles in 12 Healthy Human Subjects after Intranasal Administration of (a) 0.2 mg and (b) 0.4 mg Doses of INSCOP. Each datum point represented observed concentration from 12 subjects in Data Set 2. The solid line indicated 50th percentiles, and broken lines represented 2.5th and 97.5th percentiles from model estimations of 500 simulations.

(a)



(b)

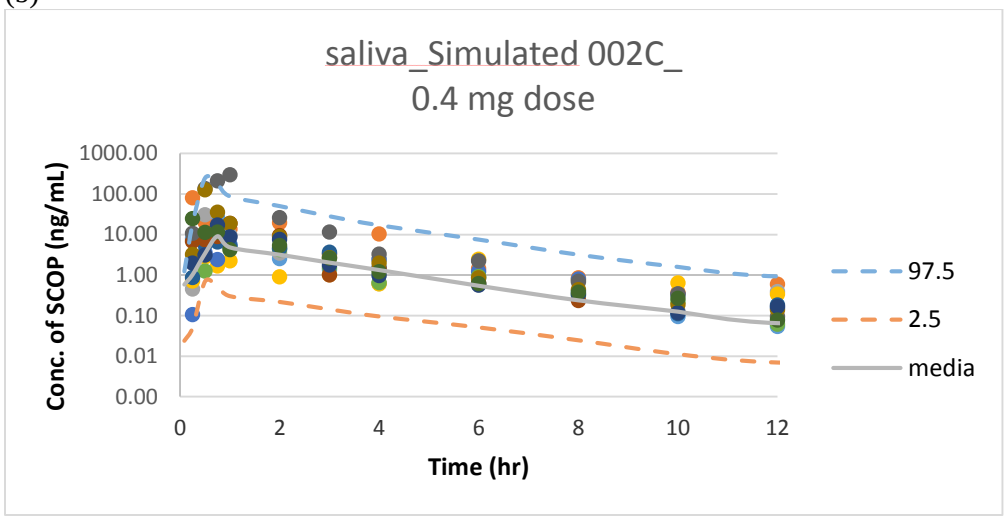


Figure 54. VPC of Saliva Concentration versus Time Profiles in 12 Healthy Human Subjects after Intranasal Administration of (a) 0.2 mg and (b) 0.4 mg Doses of INSCOP. Each datum point represented observed concentration from 12 subjects in Data Set 2. The solid line indicated 50th percentiles, and broken lines represented 2.5th and 97.5th percentiles from model estimations of 500 simulations.

Table 19. Comparison of Mean Values of Predicted Concentrations and Observed Concentrations at (a) 0.2 mg and (b) 0.4 mg Dose Levels from Data Set 2.

(a)

Time (Hour)	Observed Conc.(pg/mL)	simulated Conc.(pg/mL)	% of difference
0.00	0.00	0.00	0.00
0.25	85.22	NA	NA
0.50	193.17	168.40	12.82
0.75	284.51	NA	NA
1.00	263.58	231.50	12.17
2.00	193.60	159.50	17.61
3.00	149.63	101.80	31.97
4.00	93.83	57.20	39.04
6.00	32.38	19.70	39.15
8.00	14.25	6.12	57.05
10.00	6.01	4.21	29.93
12.00	8.84	3.67	58.49
24.00	5.23	1.25	76.08

(b)

Time (Hour)	Observed Conc.(pg/mL)	simulated Conc.(pg/mL)	% of difference
0.00	0.00	0.00	0.00
0.25	231.90	NA	NA
0.50	383.40	321.04	16.26
0.75	540.18	NA	NA
1.00	522.01	462.12	11.47
2.00	447.56	404.38	9.65
3.00	277.50	210.81	24.03
4.00	175.46	109.57	37.55
6.00	77.83	57.21	26.49
8.00	38.38	24.89	35.15
10.00	17.63	13.87	21.34
12.00	3.83	2.58	32.70
24.00	2.69	1.37	49.10

4.4.6. Compartmental Model with SCOP-G

With the validated model for SCOP, we added the SCOP-G in plasma and saliva into the model. The model structure is presented in **Figure 55**. The results of data fitting showed that there were good correlations between the observed and predicted concentrations in SCOP and SCOP-G in plasma, saliva and urine,respectively. The Log sale of goodness fit plots of SCOP in plasma, saliva and urine; as well as SCOP-G in plasma and saliva were shown in **Figure 56.** The diagnostic plot of weighted residuals versus time Profile were shown in **Figure 57**, in which, all weighted residuals fell within the narrow range from -2 to 2 and distributed around the line of 0 on Y axis in all compartments. Weighted residuals versus standard normal quantiles profiles in three compartments are presented in **Figure 58**. All weighted residuals followed the line of identity, which indicated that there were normal distribution of residuals. The diagnostic plots from PK modeling indicated that the final best fit model of SCOP and SCOP-G from INSCOP could simultaneously describe the plasma, saliva and urinary data at all subjects in all dose levels with reliability and stability.

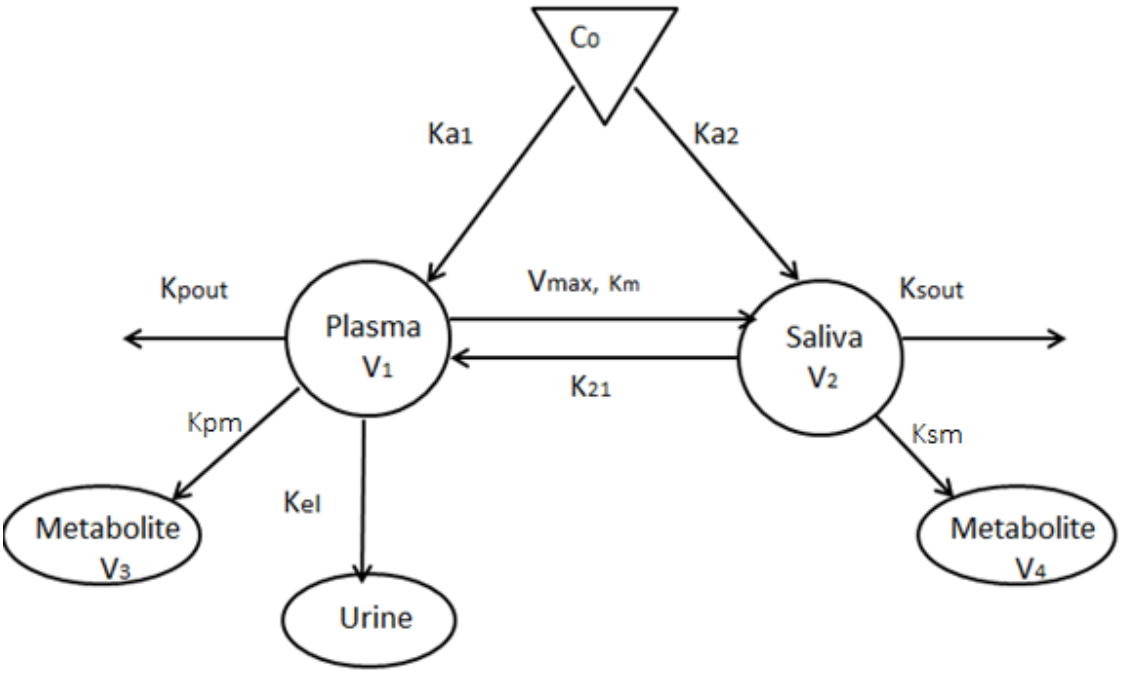


Figure 55 The Best Fit Model Structure of SCOP and SCOP-G

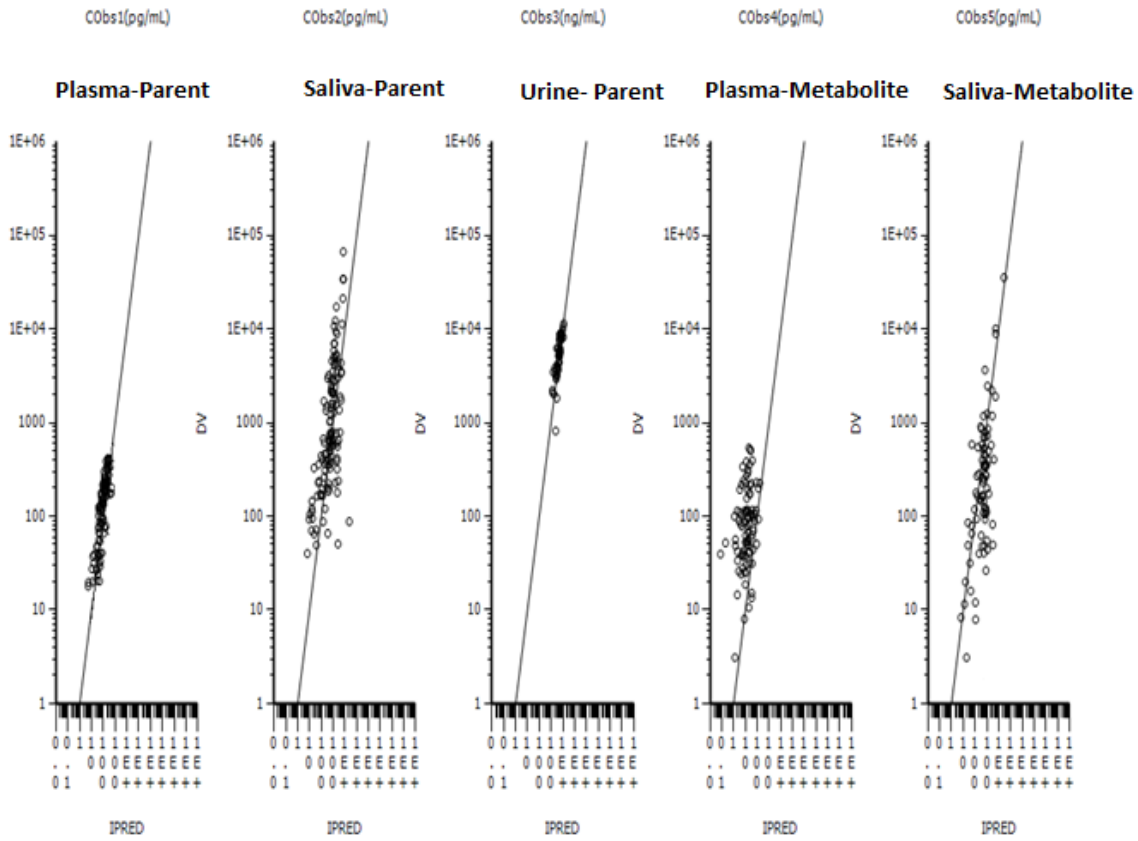


Figure 56 Log Sale of Goodness Fit Plots of Scopolamine and SCOP-G

Cobs1: observation of scopolamine in plasma;

Cobs2: observation of scopolamine in saliva;

Cobs3: observation of scopolamine in urine;

Cobs4: observation of SCOP-G in plasma;

Cobs5: observation of SCOP-G in saliva.

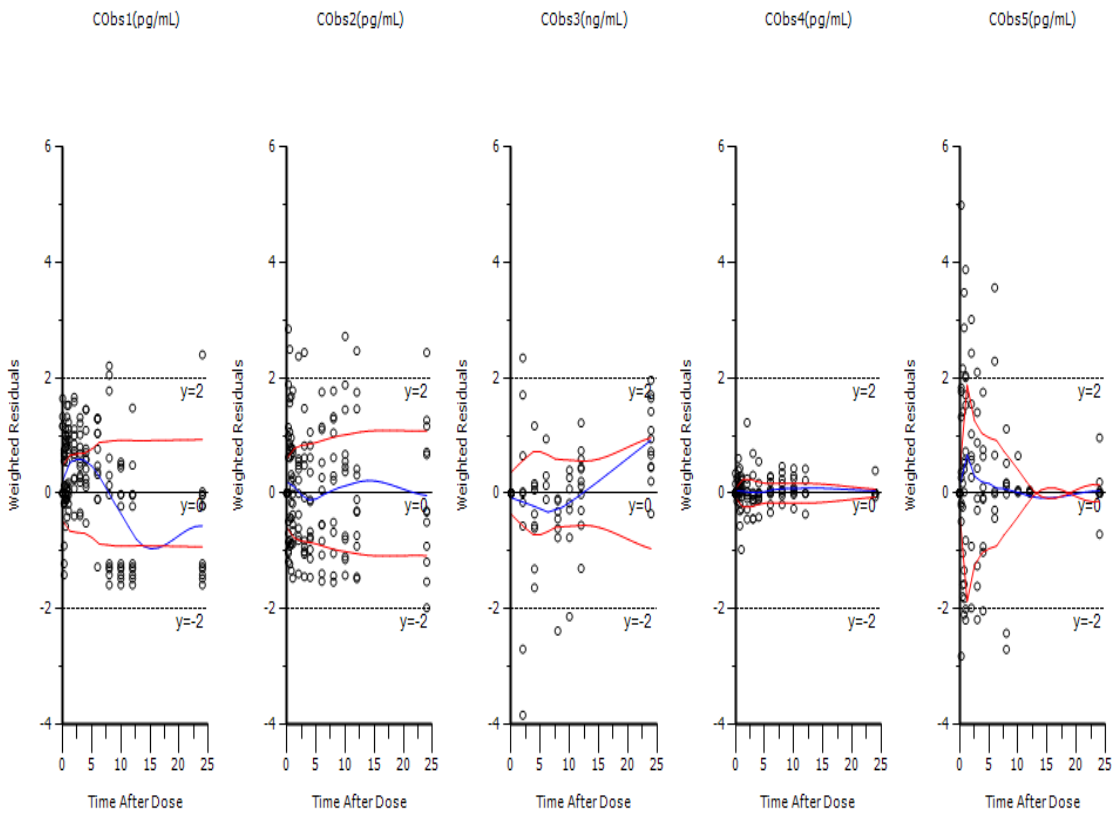


Figure 57 Weighted Residuals versus Time Profile

Cobs1: observation of scopolamine in plasma;

Cobs2: observation of scopolamine in saliva;

Cobs3: observation of scopolamine in urine;

Cobs4: observation of SCOP-G in plasma;

Cobs5: observation of SCOP-G in saliva.

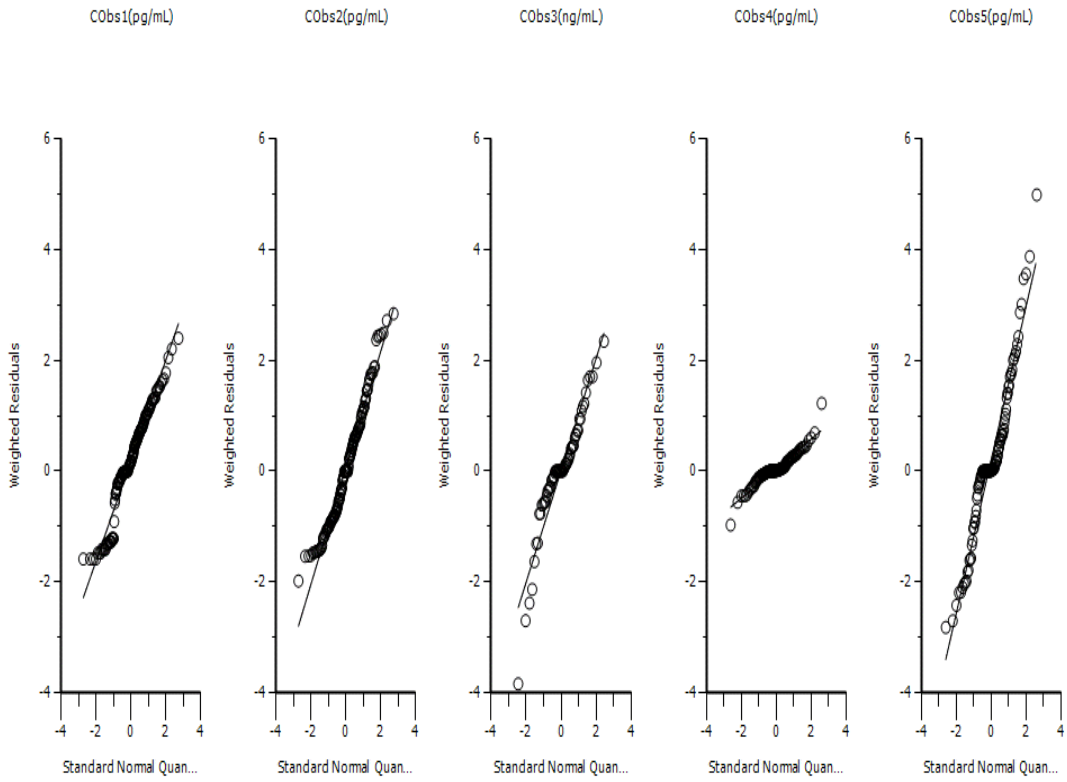


Figure 58 Weighted Residuals versus Standard Normal Quantiles Profiles

Cobs1: observation of scopolamine in plasma;

Cobs2: observation of scopolamine in saliva;

Cobs3: observation of scopolamine in urine;

Cobs4: observation of SCOP-G in plasma;

Cobs5: observation of SCOP-G in saliva.

Chapter 5 Discussion

5.1. Dose escalating Pharmacokinetics studies of INSCOP

Scopolamine is a legacy drug used for the treatment of motion sickness in the military, NASA manned spaceflight program, and in the global settings as one of the first drugs approved for marketing by the US FDA. It has a long standing safety profile established in its currently marketed formulations for multiple indications with decades of post marketing drug safety evaluations. Current doses and dosage regimens of scopolamine are considered safe although care should be exercised when taking higher than recommended doses due to detrimental side effects which include drowsiness, memory impairment, dry mouth and mydriasis.

At present, scopolamine transdermal patch is the only non-invasive formulation available on the market for the treatment of motion sickness. Doweck et al (1995) investigated the rate of scopolamine absorption through the skin and reported that therapeutic levels of scopolamine were achieved only 5 to 6 h after patch application. The main disadvantage of transdermal delivery is that therapeutic plasma levels are obtained very slowly, 6 to 8 h after application in the case of scopolamine (Price et al., 1981; Chandrasekaran et al., 1983; Clissold et al., 1985). Thus, the use of TTS-scopolamine poses a problem when immediate treatment is required. Furthermore, the transdermal administration of scopolamine also has other limitations. For example, the peak plasma concentration (Cmax) is not reached until 12–16 h after dosing. Moreover, this route provides unnecessary prolonged blood levels that result in a significant side

effect profile which includes dry mouth, drowsiness and blurred vision (Shaw et al., 1980).

Nasal administration gained attention of many pharmaceutical scientists due to its great potential utility for rapid drug delivery. It offers an attractive alternative for drugs with limited oral bioavailability that are destroyed by gastrointestinal fluids, or highly susceptible to hepatic first-pass or gut-wall metabolism (Chien et al., 1987). Results of earlier investigations indicated that while bioavailability of orally administration scopolamine was poor and variable, intranasal administration resulted in a fast, reliable and more complete absorption of the drug (Putcha, 1989 and 1996). Klocker et al. (2001) also reported that scopolamine nasal spray is an effective and safe treatment for motion sickness, with a fast onset of action within 30 min after administration. More recently, Renner et al. (2005) also reported that pharmacokinetics and pharmacodynamics of scopolamine depend on the dosage form.

INSCOP offers less variability of systemic concentrations unlike oral administration with higher concentrations of the drug achieved more rapidly due to the fast absorption (Putcha, 1996; Renner et al., 2005). The purpose of this study was to evaluate dose linearity of pharmacokinetics of this investigational new drug (IND) formulation for intranasal administration per FDA requirement of IND pharmaceutical preparations. This IND formulation is aimed at providing a rapidly acting, reliable, safe and efficacious alternative for the treatment and prevention of motion sickness experienced by astronauts in space. Other target populations may be benefited from the use of this novel formulation as well, most notably, military aviators, military and commercial seafarers. The formulation once approved for market release by the FDA is expected to facilitate administration of lower than normal therapeutic doses with no side effects in Space and on Earth.

Results of this study clearly demonstrate that INSCOP was rapidly absorbed with measurable concentrations in plasma within 5 minutes after administration of all three doses in all the subjects, reaching maximum concentrations at 1.2-1.3 h post dose (**Table 4**). Absorption and bioavailability appear to be linear at the administered dose range as indicated by Cmax and AUC (**Figures 11a and 11b**). Ratios of body weight normalized AUC and Cmax with dose (**Figures 12a and 12b**) are consistent confirming dose linear PK of INSCOP at 0.1-0.4 mg dose range (Figure2). These results indicate consistent linearity of absorption and bioavailability at all three doses, suggesting safety and reliability of treatment in this dose range without the potential for adverse side effects. Increases in both observed and derived Cmax, observed AUC at last time point along with extrapolated AUC at time infinity were also linear with dose (**Table 4**). This dose linearity of PK in addition to the short half-life (1.1h ~ 1.4h) may allow administration of multiple doses without significant side effects. Results from an earlier study by authors (Putcha, 1996) indicated that an aqueous formulation of INSCOP achieved higher Cmax and AUC values, compared to those of an equivalent oral dose. Both Cmax and AUC were higher after administration of aqueous drops compared to those in this study (1.6 ± 0.2 vs.0.3 ± 0.1 ng/ml and 2.8 ± 0.3 vs. 1.3 ± 0.6 ng*h/ml, respectively) suggesting that the aqueous formulation may offer more efficient absorption after intranasal administration. . The pH of the gel was slightly lower than aqueous drops (3.5 versus 4) which may be a reason for lower absorption from the gel since Ahmed et al (2000) reported that better bioavailability of scopolamine can be achieved by increasing the pH of nasal formulations. Simmons et al (2010) in a double blind, placebo controlled clinical efficacy trial with INSCOP using Human Disorientation Device (HDD) for inducing motion sickness in aviation candidate subjects (mean age 23.5 y), collected 4 blood samples over 1.5 h post dose to estimate drug levels to estimate drug levels associated with efficacy of treatment. They reported Cmax and

AUC values of 0.15 ng/mL and 0.1 ng*h/mL, respectively. These values are lower than those from our results (0.3 ng/mL and 1.3 ng.h/mL, respectively). However Tmax values in both studies are similar (1.3 h post dose). These results lead us to believe that both Cmax and AUC may be under estimated in their study due to lack of adequate sampling duration. Additionally, age of subject population and artificial motion environment could also contribute to differences in disposition of scopolamine in the two studies.

Urinary recovery of unchanged scopolamine and its metabolite, glucuronide conjugate (**Table 6 and Table 7**) suggest that hepatic metabolism may be an important pathway of elimination of scopolamine after administration. Cumulative amount of scopolamine and glucuronide conjugate excreted in the urine increased linier with dose. Concurrently, fraction of dose excreted as scopolamine and glucuronide conjugate in the urine were consistent and were not significantly different among the three dose levels with excretion ranging between 1.3 and 1.4 % for scopolamine and 4.2 and 5.2% for glucuronide conjugate (**Table 6 and Table 7**) . It is noteworthy that the cumulative percentage of dose excreted as scopolamine and glucuronide conjugate in the urine accounts for less than 10 % of the administered dose for all three dose levels indicating the involvement of other significant metabolic pathways for scopolamine elimination in humans. Kentala et al. (1990) reported that following incubation of urine samples with β- glucuronidase and sulfatase, total scopolamine concentrations in the urine consisting parent drug and metabolites (glucuronide and sulfate conjugates) increased almost 7 times without a significant increase in parent compound concentrations at all sample collection time points for 12 h post dosing. These results indicate that glucuronide and sulfate conjugation are important metabolic pathways of scopolamine elimination in humans. In light of the fact that only less than 5.2 % dose is excreted as glucuronide conjugate in our study, it is prudent to expect that sulfate conjugation is another significant metabolic pathway of scopolamine in human subjects. These results of overall low recovery of

administered dose in this study also support existence of other extra-hepatic metabolism pathways of scopolamine suggested by earlier reports on the elimination of scopolamine (Ebert U, Oertel R, Kirch W, 2000). No significant difference in urinary excretion rate of scopolamine and scopolamine glucuronide across sexes was observed which is in agreement with earlier reports (Ebert U, Oertel R, Kirch W, 2000).

Most pharmacodynamic studies with scopolamine thus far focused on the CNS effects of the drug. Pharmacodynamic measurements after intravenous and intramuscular scopolamine administration (0.5 mg scopolamine hydrobromide over a period of 15 minutes, and 0.5 mg in the upper right arm, respectively) indicated no interday variability of the baseline values of EEG (Ebert U, Grossmann M, Oertel R, et al., 2001). Scopolamine was reported to produce a dose- and time dependent impairment of memory and attention (Ebert U, Siepmann M, Oertel R, et al., 1998). However, all of these studies used higher dose of parenteral administration. We reported significant suppression of salivary flow rate after intranasal administration of a low dose (Putcha, 1996). A significant decrease in diastolic blood pressure after administration of intranasal scopolamine (0.4 mg or 0.2%) were also reported (Simmons et al., 2010; Klocker et al. 2001).

Limited information on the effective scopolamine concentrations for motion sickness treatment suggest that the mean scopolamine concentration in plasma required for seasickness prevention is 160 pg/ mL (Gil A, Nachum Z, et al., 2005). Recent results on efficacy of INSCOP to prevent motion sickness symptoms using motion sickness simulation device HDD suggested that 0.4 mg of intranasal scopolamine delays the onset of motion sickness and that the effect is both statistically and clinically significant as indicated by significantly more head movements tolerated by subjects after receiving

0.4 mg of intranasal scopolamine than after receiving a placebo (Simmons et al., 2010). Klocker et al (2001) also showed that scopolamine nasal spray at a concentration of 0.2% was statistically superior to both placebo and dimenhydrinate in reducing seasickness score induced by whole body vibrations by a rotating chair. Neither of these studies, however, examined pharmacodynamics of scopolamine.

The number of intranasally administered drugs approved for marketing in the US by the FDA is limited, most of which are aqueous spray formulations, and reports on dose escalation studies in humans are available for only a handful of drugs that include sublingual desmopressin (Steiner et al., 2006), intranasal insulin (Leary et al., 2006), nasal spray of zolmitriptan (Yates et al., 2002), and intranasal ganirelix (Fujimoto et al., 1997).

A gel formulation of Vitamin B12, Nascobol, was marketed by NASTECH which was subsequently abandoned and distributed at present as a spray formulation. Intranasal gel formulations are expected to provide increased retention time at the site of absorption, the nasal mucosal membrane, to enhance absorption and bioavailability compared to aqueous mists or sprays. However, gel formulations have inherent problems of inconsistent dose delivery resulting from sub-optimal specific gravity/viscosity characteristics and drug wastage due to priming volume requirements. Additionally, contamination with scopolamine during priming process of gel formulation can cause undesirable ocular side effects. Although the current gel formulation and the delivery devices performed satisfactorily in the reduced gravity environment of parabolic flight suggesting that the dosage form may be suitable for the treatment of SMS, certain aspects of formulation like higher viscosity (1945-2050 cP) and lower than optimal pH are undesirable. While the current study found desirable pharmacokinetics of escalating doses of INSCOP, irregular dose delivery and excessive priming of the pump actuator

required on occasions must be addressed for optimizing performance and efficacy of INSCOP.

In conclusion, INSCOP offers an attractive therapeutic alternative for motion sickness in light of its reliable and efficacious delivery characteristics in addition to being a simple and cost-effective formulation of an age-old drug with long standing safety record for use in modern medicine. It is expected that intranasal administration can provide higher concentrations more rapidly than oral doses that can enhance speed of onset and duration of therapeutic activity. Further, since nausea and vomiting are common symptoms of motion sickness, which can hinder absorption from an oral dose, exasperated by poor bioavailability due to extensive first pass metabolism after oral administration, it is hoped that treatment of motion sickness both on Earth and in space can be efficaciously managed before and after the onset of symptoms. Presently, clinical trials are underway with a modified aqueous spray formulation that will offset some of the aforementioned disadvantages encountered with the gel formulation.

5.2. Sex impact on PK of INSCOP in plasma

Sex differences in PK parameters were observed in high dose level of 0.4 mg during individual PK analysis. The population PK analysis also indicated that sex-dependent pharmacokinetics of SCOP at the high dose level of 0.4 mg. With covariate analysis of population PK modeling including sex, age and body weight as covariates, sex is confirmed to be a significant covariate on population PK of SCOP from INSCOP in Vd and Cl.

With respect to sex differences in individual analysis of pharmacokinetics of SCOP, Ebert et al (2000) reported that C_{max} was higher in males than in females after intravenous infusion of 0.5 mg dose for 15 minutes, suggesting that females may need higher doses than males to achieve the minimum effective concentration. Results from our study showed sex differences in some of the parameter estimates only at the high dose of 0.4 mg. Mean C_{max} was lower in males than in females ($p<0.05$), concurrently, clearance and volume of distribution values in males were higher than those correspondingly in females ($p<0.05$). However, no significant difference in the AUC between males and females was detected by Wilcoxon rank-sum (Mann-Whitney) test ($p=0.08$), resulting from the highest degree of variability observed with dose dispensed for 0.4 mg treatment with a mean of 0.5±0.3 mg ranging between 0.3 and 1.2 mg. Men and Venitz (2004) also reported similar sex differences in volume of distribution and half-life with males having a 45% higher V_{dss} and a 26% longer t1/2 than females after a 6.7 mg/Kg intravenous dose. While these authors also reported significant age differences in clearance and volume of distribution with elderly subjects having higher values than those in the young subjects, attributable to changes in plasma/tissue protein binding and/or extra-hepatic metabolism. No age-related difference in AUC was observed in our

study when subjects were divided into two age groups of 21-29 and 33-47 years. Age range for subjects in our study was intentionally kept narrow (21-47 years) in order to match the age distribution of astronaut population.

The reported sex differences in the pharmacokinetic parameters of scopolamine in this study may be attributed to possible physical and physiological differences between sexes, e.g. total body weight, plasma volume, blood flow which could influence volume distribution and clearance, and possible differences in entero-hepatic metabolizing enzymes and protein binding. According to the reviews of the Adverse Events Reporting System (AERS) from the United States Food and Drug Administration (US FDA), women experience more adverse events than men, which implies that at a given dose a drug reaches higher free drug concentrations or remains longer in the body in females than in males. Data from CDC (Advance Data No. 347 October 27, 2004) shown that the total body water, extracellular water, intracellular water, total blood volume, plasma volume, and red blood cell volume are greater for men than women. The greater total body water, plasma volume, extracellular water, and intracellular water will increase the volume of distribution thus decreasing drug concentration which explain the observation of sex differences at 0.4 mg dose of INSCOP in our study.

Sex-dependent differences in metabolism may be another reason for PK difference between sexes. Some specific drugs such as nicotine, chlordiazepoxide and flurazepam shown the sex-dependent differences in metabolism (Greenblatt DJ et al., 1997; Cooper SF et al., 1984). Scopolamine is mainly metabolized by Phase II enzymes UDP-glucuronosyl- transferases and Sulfo- transferases, The activities of these two enzymes are higher in male then in female; at the same time, a hepatic blood flow are lower in women than in men normalized per m^2/kg (Anderson GD, 2002). These factors can cause significantly higher plasma concentrations in women, and higher volume of distribution and clearance in male after administration of INSCOP at 0.4 mg dose.

In the population PK analysis of SCOP from INSCOP, based on observed plasma data from 12 healthy human subjects, sex, age and body weight were selected to perform covariate analysis. To our knowledge, this is the first population covariate analysis study report of INSCOP. Our findings of significant effect of sex on CL and Vd of SCOP from INSCOP were consistent with the finding in our previous individual analysis. During the population analysis, we chose to use one compartment model structure with first order elimination, while Marieke et al. (2011) used a two compartment model to describe the PK of scopolamine after 0.5 mg of I.V. administration for PK/PD population analysis. Base on the Semi-log profile and goodness fit plot, as well as the diagnostic plots from our results, the INSCOP has a short half-life with a one elimination phase, indicating that the one compartment model could best describe our PK data.

During the population PK analysis of INSCOP in our study, the combined additive and proportional residual error model was chosen. Based on our dense pharmacokinetic data from 12 subjects, this error model was suitable because it broadly reflected assay variability. In the covariate analysis, the stepwise approach was employed to select the covariates. The criterion was set for -2LL >3.84 and the P-value at 0.05 for the consistency with individual analysis. The results indicated that only sex has a significant impact on clearance and volume of distribution of SCOP from INSOP.

In conclusion of sex impact study, both individual and population analyses of SCOP from INSCOP indicated the sex difference in pharmacokinetics of INSCOP at 0.4 mg dose. Attention has to be placed in dose adjustment between sexes for astronauts when high dose (0.4 mg) of INSCOP is administrated.

In general, data on sex differences are mostly obtained by statistical analysis with small sample size and, therefore, the conclusions that can be drawn are limited. For a better understanding of the basic mechanisms of sex differences, future studies should be

designed on INSCOP. More specific data will help to determine the extent to which these differences will have implications for clinical management.

5.3. Relative bioavailability study in healthy human subjects under ambulatory and simulated microgravity conditions

The systemic exposure and relative bioavailability of 0.2 mg and 0.4 mg dose of INSCOP during ambulation (AMB) and simulated microgravity, Antiorthostatic Bed Rest (ABR) were determined and compared in this study. The result indicated that the simulated microgravity condition had no effect on the PK behavior of SCOP from INSCOP.

The study of PK of INSCOP in space is crucial but the opportunities of clinical research in space are limited. Early studies showed that antiorthostatic bed rest (ABR) elicits some of the early physiological effects of microgravity including rapid fluid shifts and cardiovascular changes (Kakurin et al., 1976; Blomquist et al., 1980). Putcha et al. (1989, 1991) revealed that the absorption and bioavailability of SCOP decreased significantly after an oral administration under ABR condition. This may result from the changes in GI function associated with microgravity. The distribution and elimination of intravenous scopolamine are not different during ABR compared with AMB control in this study. In our study, after intranasal administration of two doses of SCOP, the pharmacokinetic analysis indicated that no differences were observed on all PK parameters between AMB and ABR groups at all doses. Our results indicated that the intranasal administration of SCOP avoids the first pass effect; the GI motility changes caused by microgravity have no effect on drug absorption and the ABR may not affect metabolism and/or clearance of INSCOP.

5.4. Compartmental pharmacokinetic modeling

In this study, a complex compartmental PK model was developed to describe the SCOP concentrations in plasma, saliva and urine. One compartment each of plasma, saliva and urine were linked with different rate constants, and non-linear PK was employed to describe the drug distribution from plasma to saliva. The PK model describing first-order excretion into urine or a nonlinear renal clearance has already been reported (Thompson GA, and Toothaker RD, 2004). There is no reported PK model of SCOP describing the plasma and saliva concentration-time profiles using a saturable kinetics of drug distribution into saliva. The behavior of drug salivary elimination has been reported as first order kinetics in previous studies for different drugs (Scott et al., 2009; Nynke Teeninga et al., 2013).This proposed and validated PK model can be used to accurately predict the plasma concentration of SCOP using saliva samples which are easier to collect non-invansively by astronauts in the space.

The partition coefficient study between saliva and plasma compartment demonstrated that the equilibrium K value decreased with the dose increasing from 0.1 mg to 0.4 mg for both SCOP and SCOP-G. Saturation of drug distribution process from plasma to saliva was indicated and non-linear PK was used to describe the kinetics between plasma and saliva compartments. The diffusion of drug to saliva depend on five factors: Molecular mass; Lipid solubility; Degree of ionization; salivary pH-value and protein binding (Rainer Haeckel et al., 1996). There were three major mechanisms of drug transporting from plasma to saliva via epithelia cell including passive transcellular diffusion, ultrafiltration and active transport (Landon and Mahmod, 1982). Base on the physicochemical properties of SCOP, the potential mechanisms of drug distribution to

saliva for SCOP and SCOP-G would be passive diffusion and active transport. The non-linear PK of SCOP transfer from plasma to saliva could be due to the saturation of certain transporters involved in the SCOP transport through epithelia cell membrane.

The main objective of this study was to develop a PK model that could describe the PK profiles of SCOP including excretion data such as the salivary and urinary excretions of unchanged SCOP and SCOP-G. During the optimization of model structure for SCOP, several over-parameterized models (i.e., **Figure 39**) were tested to describe the SCOP in plasma, saliva and urine. Such a model resulted in one or more parameters with high imprecisions, because there was insufficient sets of data to estimate the derived number of parameters. Meanwhile, in individual analysis of urine data of 12 subjects, the cumulative percentage of dose excreted as SCOP and SCOP-G in the urine accounts for less than 10 % of the administered dose. Therefore, we concluded that renal excretion was not a major elimination pathway and SCOP-G is not the major metabolite for SCOP. As a result, we simplified our model to a one-compartment model for plasma saliva and urine linked with different rate constants for parent drug only. The metabolisms of SCOP in plasma and saliva were expressed by the parameter Kpout and Ksout (Model B, **Figure 45**). After the inclusion of non-linear PK for SCOP transfer from plasma to saliva compartments, the best fit model was developed and model discrimination was performed on data using Phoenix, by minimizing the Akaike Information Criteria (AIC) and by comparison of the quality of fit plots.

With the best fit model of SCOP, good correlations between observed and predicted concentrations were obtained in plasma, saliva and urine compartments. The PK parameters described in the model (Model C) were derived from 12 subjects. The CV% of PK parameter estimates shown in **Table 15** revealed the large variation of 12 human subjects which reflected the reality in the data of clinical studies. The PK parameter Kel which describes the drug elimination to urine were 0.01 hr^{-1} at the three dose levels. The

low Kel was in an agreement with urine excretion from individual analysis, demonstrating that kidney was not a major elimination route for SCOP. The Km is the Michaelis Manton constant for excretion of SCOP into saliva, and the values were 137.2, 127.7 and 158.3 pg/mL for 0.1, 0.2 and 0.4 mg doses, respectively. The Km values indicated that after the concentration of SCOP reaches the Km value, the maximum velocity (V_{max}) for excretion into saliva reaches its half value. The mean value of C_{max} observed in 12 subjects after 0.1 dose were 90 pg/mL, and increased to 150 and 330 pg/mL after 0.2 and 0.4 mg doses. The non-linear increase in C_{max} were in an agreement with the results of partition coefficient ratio study that revealed nonlinear PK after dose increase to 0.2 and 0.4 mg, respectively. Saliva suppression was reported in previous studies with oral or transdermal administrations of SCOP (Markkanen et al. 1987;Giulio Pagliuca et al., 2012).In our study, the volumes of distribution (mean with CV %) in saliva were 41.5 (87.2%), 37.4(56.1%) and 29.3 (35.4%) L after 0.1, 0.2 and 0.4 mg doses, respectively. The mean Vd in saliva reflected that there was a trend of reduction in saliva secretion. The best fit model was validated with internal and external validation approaches. The purpose of model validation is to examine whether the model could offer a good description of the validation data. The results of visual prediction check (VPC) from both internal and external validations confirmed the stability and reliability of the PK model. All observed plasma and saliva concentrations of SCOP were within the 95% CI, except a few high concentrations in plasma from external data in Specific Aim 2. The INSCOP used in the Aim 2 study was from another vendor, different from that of INSCOP for Aim 1 study. The dose variations for INSCOP might be accounted for the high concentrations in plasma of Aim 2 study.

There were several limitations with this study. First, only 24 subjects were employed in this study, among which 12 subjects were used to perform data fitting and model building. Others 12 subjects were used to perform validation. A large sample sizes of

subject population would have enhanced the predictive performance of our results. The second limitation was that the metabolite we quantified was not the major metabolite for SCOP in humans. The urinary compartment in the best fit model was not as useful as saliva compartment. Further studies focusing on SCOP metabolism should be conducted and the mechanism of SCOP elimination should be clearly demonstrated in the future.

In conclusion, the PK model describing SCOP concentrations was developed and for the first time it satisfactorily estimated the PK of INSCOP in plasma, saliva and urine. The non-linear PK was described in the best-fit structural model for SCOP transfer from plasma to saliva compartments. The inclusion of non-linear PK resulted in a significant improvement in model fitting. The model can be utilized to predict the INSCOP plasma concentrations by saliva concentrations. The non-invasive sampling of saliva would be useful for the assessment of PK or dosing of SCOP in space and other remote environments, without requiring invasive blood sampling.

Chapter 6 Summary

This project has a significant clinical implication in understanding and predicting PK of intranasal scopolamine on Earth and in Space. We have demonstrated that (1) absorption and bioavailability appear to be linear within the administered dose range (0.1~0.4 mg) of intranasal scopolamine; and (2) sex is a significant covariate for population pharmacokinetic model of INSCOP, which indicates that sex-dependent pharmacokinetics of scopolamine at the high dose level of 0.4 mg. We also demonstrated that (3) absorption and relative bioavailability are not significantly affected in the simulated microgravity condition. We have also shown that (4) PK model for INSCOP is developed and for the first time satisfactorily estimated the PK of INSCOP in plasma, saliva and urine. Saliva samples can be used to predict plasma concentrations of INSCOP, which will be useful for the assessment of PK or dosing of scopolamine in space.

6.1. Dose escalating Pharmacokinetics studies for space motion sickness

A phase II clinical trial was designed and approved by the NASA Johnson Space Center Committee. Twelve healthy human subjects were recruited in a dose escalating study from

0.1, 0.2 to 0.4 mg doses. The plasma concentration-time profiles of INSCOP as well as scopolamine glucuronide conjugate in healthy human subjects were established at three dose levels. The pharmacokinetic parameters were derived from WinNonlin using 1-compartment PK model. The result indicated that the absorption and bioavailability are linear within the administered dose range (0.1~0.4 mg) of intranasal scopolamine

6.2. Sex impact on PK of INSCOP in plasma

Individual and population PK analyses of INSCOP were employed to study the sex impact on PK of INSCOP. Twelve healthy human subjects participated in this study and plasma-time profiles were obtained. Sex, body weight and age were employed as covariates in the population PK analysis. Individual PK results indicated that C_{max} in females was higher than that in males, resulted from a faster clearance and larger volume of distribution in males than in females. The results from population PK analysis indicated that the volume of distribution and clearance are significantly higher in males than in females, and sex was a significant covariate to the pharmacokinetic model of INSCOP at the dose of 0.4 mg.

Therefore we conclude that sex-dependent pharmacokinetics of scopolamine at the high dose level of 0.4 mg, and more attention should be paid to dose adjustment between sexes for astronauts when high dose of 0.4 mg of INSCOP is administrated in space.

6.3. Relative bioavailability study in healthy human subjects under ambulatory and simulated microgravity conditions

A Phase II, randomized, double-blind, bioavailability study of intranasal scopolamine in a simulated microgravity environment was performed with two dose levels (0.2 and 0.4 mg) in 12 normal healthy subjects (6 male/ 6 female). The plasma concentration-time profiles of INSCOP were established. Concentrations of SCOP were measured in plasma by a validated LC-MS/MS method and pharmacokinetic parameters were derived from WinNonlin using one-compartment model. The results indicated that the simulated microgravity, antiorthostatic bed rest (ABR) condition does not alter the pharmacokinetics of INSCOP at the two dose levels (0.2 mg and 0.4 mg).

6.4. Compartmental pharmacokinetic modeling

From the clinical studies, we had twenty-four healthy subjects in ambulatory condition. The concentrations of SCOP from INSCOP were measured in plasma, saliva and urine by the validated LC-MS/MS method. PK modeling and data fitting were performed using data from specific aim 1 and model validation were performed using data from specific aim 2. The best fit structural model for INSCOP was developed, consisting of one compartment each for plasma, saliva and urine, connected with processes of different rate constants. Nonlinear PK of INSCOP was employed to describe the transfer kinetics of SCOP from plasma to saliva compartments. PK parameters were derived from the model and model validation were successfully performed by internal and external validation approaches. The results shown that the model can first time satisfactorily derived the PK of SCOP from INSCOP simultaneously in plasma, saliva and urine. Saliva samples can be used to

predict plasma concentrations of SCOP from INSCOP. The non-invasive saliva sampling can be useful for the assessment of PK or dosing of scopolamine in the space.

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