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DEVELOPMENT OF A NOVEL APPROACH TO ASSESS QUALITATIVE AND QUANTITATIVE DYNAMICS ASSOCIATED WITH THE SUBCUTANEOUS OR INTRAMUSCULAR ADMINISTRATION OF PHARMACEUTICALS AND ASSOCIATED PARENTERAL DELIVERY SYSTEMS

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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Dedication

This dissertation is dedicated to my family; including my wife, Autum, and my children Avryn, Copelan, and Ellisyn; my parents, Gary and Linda; my brothers, Evan, Jeff and Byron; as well as my colleagues at Intellijject. Their support and encouragement have enabled me to reach higher, dream bigger, and accomplish more than I ever could have imagined.

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I thank my loving wife, Autum, for being so understanding and reassuring throughout this challenging journey. I thank my children, Avryn, Copelan, and Ellisyn for being at the door each time I would come home for a much-needed hug and for reminding me of how truly blessed I am to have such a beautiful and supportive family.

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Finally, I thank my friends, mentors, and colleagues at Intelliject for their unwavering dedication to patients and for allowing me the opportunity to expand my knowledge in the pharmaceutical sciences. It has been quite a journey and the story continues, one chapter at a time.

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Abbreviations

≥	greater than or equal to
≤	less than or equal to
∞	infinity
®	registered trademark
™	trademark
%	percent
AUC	area under the curve
AUC_{0-t}	area under the concentration-time curve between time zero extrapolated to the last sampling time point
$AUC_{0-\infty}$	area under the concentration-time curve between time zero extrapolated to infinity
$AUC_{0-partial}$	area under the concentration-time curve determined at various early exposure time points (AUC_{0-30}, AUC_{0-40}, AUC_{0-60}, and AUC_{0-Tmax})
AUC_{0-Tmax}	area under the concentration-time curve between time zero extrapolated to the time maximum plasma concentration was reached
bmp	beats per minute
C	concentration
°C	degrees Celsius
CI	confidence interval

C_{max}	the highest concentration on the plasma-concentration time profile
cm	centimeter
CRSU	Clinical Research Center
CS	Clinically Significant
CT	Computed Tomography
DSMB	Data Safety and Monitoring Board
ECG	electrocardiogram
e.g.	exempli gratia (for example)
FDA	Food and Drug Administration
GFR	glomerular filtration rate
g	gram
h	hour
HIV	human immunodeficiency virus
hrs	hours
HU	Hounsfield unit
ID	Intradermal
i.d.	internal diameter
i.e.	id est (that is)
IM	Intramuscular
IV	Intravenous
ka	first-order rate constant for absorption into systemic circulation
kg	kilogram

ln	natural logarithm
LOQ	limit of quantitation
$Loss_{Tiss}$	Iohexol loss rate from the extravascular tissue into systemic circulation
m^2	meter-squared
mg	milligram
mgI	milligrams of Iodine
min	minutes
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
µ	micro
MRI	magnetic resonance imaging
mSv	millisievert
n	number of data
NCS	not clinically significant
nm	nanometer
r	correlation coefficient
s	seconds
SOP	standard operating procedure
SC	subcutaneous
SD	standard deviation
SE	standard error

SPECT	single-photon emission computed tomography
Sv	sievert
t	time
T_{last}	the last observed plasma concentration
T_{max}	time to reach highest concentration (Cmax)
$T_{1/2}$	half-life
US	United States
v/v	volume by volume
VCU	Virginia Commonwealth University
vs.	versus

Abstract

DEVELOPMENT OF A NOVEL APPROACH TO ASSESS QUALITATIVE AND QUANTITATIVE DYNAMICS ASSOCIATED WITH THE SUBCUTANEOUS OR INTRAMUSCULAR ADMINISTRATION OF PHARMACEUTICALS AND ASSOCIATED PARENTERAL DELIVERY SYSTEMS

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There has been a significant increase in the number of injectable pharmaceutical products over the last decade that have been incorporated into unique delivery systems such as pen injectors, auto-injectors, or pre-filled syringes. The advancement of these delivery systems and the paradigm shift towards administration of injectables in the out-of-hospital or home setting have introduced variables that can affect the bioavailability of injectable drugs and potential pharmacologic outcomes. An approach that allows for the qualitative and quantitative dispersion assessment of an injectable at the moment of tissue deposition coupled with an assessment of systemic exposure parameters could provide substantial information to researchers developing new injectable formulations and associated delivery systems.

The overall goal of this research project was to develop an approach for investigating various injection dynamics, more specifically, dispersion dynamics associated with the

administration of parenteral pharmaceutical products utilizing delivery technologies designed to deliver drug below the dermis. This was accomplished by first evaluating the safety and usability of computed tomography (CT) scanning as a novel radioimaging approach to assess qualitative and quantitative dispersion parameters in a cadaver study followed by a randomized, controlled, clinical study to assess CT tissue dispersion and the systemic exposure of iohexol, administered subcutaneously by two delivery systems in human volunteers.

The primary finding of this work was the demonstration that CT scanning may be combined with a systemic exposure assessment to provide an effective paradigm for investigating dynamics of injectable delivery impacted by a variety of factors, including the choice of delivery system. In this study, iohexol delivered subcutaneously by an auto-injector resulted in notable qualitative and quantitative dispersion differences, including a higher rate of iohexol loss from the extravascular tissue, as well as differences in early plasma exposure as compared to a pre-filled syringe delivery system. The injections and CT scanning were well tolerated with adverse events limited to mild injection site reactions resolving without intervention. This research resulted in a novel local *in-vivo*$_{\text{(extravascular disappearance)}}$, systemic *in-vivo*$_{\text{(intravascular appearance)}}$ correlation approach that could be utilized to assess a wide variety of dynamics associated with injectable drug delivery below the dermis.

CHAPTER 1. INTRODUCTION

Background

Pharmaceuticals may be administered into the body locally or systemically through a variety of administration routes utilizing a variety of dosage forms. The oral and injectable routes of administration dominate the prescription pharmaceutical market, accounting for more than 80% of pharmaceutical sales each year (PharmaVitae, 2009). However, as companies continue to lose patent protection on many of their blockbuster oral therapies, coupled with a lack of oral compound research and development pipeline productivity, novel injectables are projected to drive overall growth in the pharmaceutical and biotechnology industries through 2014 (Datamonitor, 2009). Over 95% of biotechnologically derived drugs are injectable products (PCMO, 2005). In years past, many of these biotech drugs were developed for small populations with rare diseases which could be treated in an affordable manner. However, new injectable medicines have emerged that are for more common, chronic diseases including asthma, diabetes, and arthritis. Over 50% of these injectables are now utilized in drug delivery technologies such as pre-filled syringes, pen injection systems or auto-injectors (Zitter, 2008). In addition, due to, in-part, cost pressures on the healthcare system, many of these drug products that are incorporated into delivery technologies have been approved for self or caregiver administration in the out-of-hospital setting, especially through intramuscular (IM) or subcutaneous (SQ) routes.

This is because there are substantial costs savings in reducing regular clinic or office visits for healthcare provider administration of injections. By 2012, it is estimated that over 12 million Americans will be utilizing some type of specialty injectable drug at home to manage their acute or chronic disease (Nagle, 2005).

Parenteral routes of drug administration such as injection into the IM or SQ tissue are as useful and important as other traditional administration routes, including the oral and inhalation routes. IM and SQ injections are administered via the extravascular system where the drug must leave the site of injection to enter the systemic circulation in order to distribute throughout the body and produce the desired pharmacological response. Because extravascularly administered drugs must traverse several barriers to reach the systemic circulation and/or the site of action, there are various factors that can affect the rate of drug elimination from the injection site. Conventionally, pharmaceutical companies attempting to model and conduct research on dynamics associated with the bioavailability of injectables have focused on factors associated with properties of the drug and the formulation. These include drug concentration, physicochemical characteristics of the active ingredient and/or excipients, and injection volume, among others. However, the advancement of novel delivery systems and the paradigm shift towards administration of injectables in the out-of-hospital or home setting has introduced other dynamic variables that could affect the bioavailability of injectable drugs and potential pharmacologic outcomes. Variables including injection device mechanics (including needle length, needle gauge and the kinematics of injection), administration factors (such as reproducibility of patient, caregiver, or healthcare provider injection technique) and certain unappreciated physiologic factors (such as subcutaneous or muscle tissue thickness and inter-

individual blood flow site differences) may be as important as those dealing with the injectable formulation themselves and warrant investigation. As a result, there is a need to develop novel approaches to assist in understanding tissue dispersion and systemic exposure dynamics that may be introduced and associated with these additional variables.

Specific Aims

Aim 1. To evaluate Computed Tomography (CT) as a potential approach for investigating sources of variability in injectable systems and to assess the dispersion of an injectate beneath the dermis over time with its relative bioavailability using iohexol, a type of radiocontrast media (RCM), as an injectable standard.

Aim 2. To assess the discriminatory capacity of CT for predicting systemic exposure by investigating the inter-individual and intra-individual variability in injectable dispersion using two distinct delivery systems.

Hypotheses

Utilizing two FDA-approved delivery systems, it is hypothesized that Computed Tomography Scanning following the administration of an imaging agent, Iohexol, and its associated assay will result in qualitative and quantitative data that will be useable to construct an approach to assess variables associated with injectable drug delivery and associated delivery systems. It is also hypothesized that there will be a difference in iohexol dispersion between the two delivery systems.

Significance

There has been a significant increase in biotechnologically-derived, injectable pharmaceutical products over the last decade that have been incorporated into unique delivery

systems such as pen injectors, auto-injectors, or pre-filled syringes for self or caregiver-administration. Due to the high-cost and complex nature of many of these injectable products, a failure in the delivery of these drugs to a patient may have significant therapeutic and cost implications.

In-vivo models have been developed to assess exposure dynamics at the absorption site in the oral, inhaled, and transdermal drug delivery routes; however, *in-vivo* predictive models or approaches to assess dynamics associated with parenterally administered products at the moment of tissue deposition are lacking. Moreover, although some *in-vitro* models exist that can assess some of the aforementioned dynamics, there are no approaches *in-vivo* that specifically assess deposition or dispersion of an injectable and the resultant systemic exposure variability that may be introduced by different injectable delivery systems or that may be introduced by different delivery techniques.

Therefore, there is a need for investigations that result in a practical paradigm that may be utilized to assess non-traditional dynamics of injection introduced before systemic exposure can be measured. The overall goal of this project is to develop an approach for assessing various injection dynamics, more specifically, dynamics associated with the administration of parenteral pharmaceutical products utilizing delivery technologies below the dermis.

Successful realization of the project aims will result in data to support further development of an approach that may efficiently and effectively investigate a variety of delivery systems and associated injectable pharmaceutical products. This could be used by pharmaceutical companies undertaking research and development of parenteral products indicated for administration in a delivery device (pre-filled syringe, pen injector, or auto-injector)

and/or by academic and other pharmacotherapeutic research scientists seeking to provide information on these and other dynamics that may improve the health and well-being of patients.

CHAPTER 2. BACKGROUND AND REVIEW OF THE LITERATURE

Factors Affecting the Systemic Exposure of Intramuscular or Subcutaneously Administered Drugs

Introduction

Injection of a drug product intramuscularly or subcutaneously leads to the initiation of events that collectively make up the absorption process. From the relatively small, localized region where a drug depot is established following injection, a pharmaceutical product is absorbed into the bloodstream or lymphatic circulation (for high molecular-weight molecules) by means of physical penetration and permeation processes that are associated with passive diffusion and partitioning through the capillary membrane. Eventual drug absorption into the bloodstream is influenced by several physicochemical variables that affect diffusion.

The absorption of a pharmaceutical product into the bloodstream may be influenced by factors including:

- the nature of the pharmaceutical product itself,
- physiologic differences between subjects,
- anatomical variations between subjects,
- variability associated with the mechanics of administration by a patient or care provider, and
- variability associated with the delivery system or vehicle.

Each of these factors play a role in the rate and extent of injectable drug absorption and are discussed further below.

Pharmaceutical Product Factors

Drug solubility and *pH.* Regardless of the dosage form administered into the subcutaneous or intramuscular tissue, a drug must eventually be in solution in an aqueous system for it to be exposed to the processes that will eventually result in its absorption into the bloodstream. Therefore, ultimately, only the fraction of drug in solution is available for absorption. Differences in the *pH* of the administered drug product and the physiological pH at the injection site may also result in solubility changes that may affect the absorption of the drug. Therefore, many drug products have their *pH* adjusted with either HCL or NaOH in part to help control the solubility of the active ingredient and subsequent absorption profile (increasing or decreasing the time to absorption).

Passive diffusion. The rate of passage of a drug through a biological membrane by passive diffusion is affected by several physicochemical factors such as the concentration gradient, partition coefficient, degree of ionization, macromolecular binding, and osmolality of the drug product.

(a) Concentration gradient. The rate at which a drug injected into the extravascular space crosses a semi-permeable membrane by passive diffusion is described by Fick's Law, expressed as:

$$\frac{\partial Q}{\partial t} = \frac{DA\left(C_1 - C_2\right)}{h}$$

where:

$\partial Q / \partial t$ = The amount of transfer of the drug per unit of time,

D = the diffusion constant

A = the surface area that is available for diffusion

C_1 = concentration of the diffusing pharmaceutical in the extracellular fluid compartment

C_2 = concentration of the diffusing pharmaceutical in the intracellular fluid compartment

h = thickness of the membrane

The magnitude of the diffusion constant is influenced by the physicochemical properties of the drug molecule and the characteristics of the membrane. Once the drug unidirectionally passes through the biological membrane, it is immediately distributed by the circulation (or lymph). For example, in the case of intramuscularly-administered pharmaceuticals, C_1 is always much greater than C_2, establishing a "sink" condition and effectively reducing Fick's equation to:

$$\frac{\partial Q}{\partial t} = \frac{DC_1}{h}$$

(b) Partition coefficient. The partition coefficient is the ratio of concentrations of a compound in the two phases of a mixture of two immiscible solvents at equilibrium. Hence these coefficients are a measure of differential solubility of the compound between two solvents (one water, the other hydrophobic). Therefore, the partition coefficient is a measures of how hydrophilic ("water loving") or hydrophobic ("water fearing") a chemical substance is. Hydrophobic drugs with high partition coefficients are preferentially distributed to hydrophobic compartments such as lipid bilayers of cells while hydrophilic drugs (low partition coefficients) preferentially are found in hydrophilic compartments such as blood serum. As an example, in the case of intramuscular or subcutaneous absorption, drugs with the higher partition coefficient will be absorbed by passive diffusion and distributed faster than water-soluble drugs with a low partition coefficient.

(c) Degree of ionization. Ionization has a profound effect on the absorption of drugs. The degree of ionization of an acid or base is determined by an ionization constant, *pKa* and the *pH* of the drug product. Virtually all drug-like molecules are weak acids or bases. This means that they contain at least one site that can reversibly disassociate or associate a proton (a hydrogen ion) to form a negatively charged anion or a positively charged cation. Molecules that disassociate protons are acids, and those that associate protons are bases. The reversibility, means that a sample is always in an equilibrium with some fraction protonated and the rest deprotonated:

$$HA \Leftrightarrow H^+ + A^- \text{ or } HB^+ \Leftrightarrow H^+ + B$$

By varying the availability of protons (i.e. the acidity of the drug product) the balance of the equilibrium can be shifted. This provides a measure of the ease of proton disassociation of a site in a compound, the disassociation (or ionization) constant *pKa*, defined by the equation:

$$pKa = pH + \log(\text{protonated}/\text{deprotonated})$$

Alternatively, the *pKa* of a site can be thought of as the *pH* at which the protonated and deprotonated fractions are equal. If the *pH* is higher than the *pKa*, the site is mostly deprotonated, and if the *pH* is lower than the *pKa*, the site is mostly protonated. Therefore, the degree of disassociation of a compound at physiologic *pH* affects the amount of partitionable nonionized weak acid or weak base species available for absorption from the injectable dosage form.

(d) Macromolecular (or protein) binding. All biologic fluids contain macromolecules as proteins that have an affinity for certain drugs. In general, these proteins are usually too large to pass through a membrane by filtration or passive diffusion. Therefore, when a drug becomes

complexed with a protein (such as albumin), its effective free or "diffusible" form becomes lowered, reducing the rate of passive diffusion. Protein binding has a significant effect on passive diffusion when the drug is bound by more than 90% because the desorption rate from the drug-protein complex will be slower than the diffusion rate (in most circumstances) of the drug through biological membranes.

(e) Osmolality. Most parenteral products for intramuscular or subcutaneous administration are formulated to be isoosmotic with tissue fluid in order to reduce the possibility of irritation that can result if osmotic differences between tissue fluid (or red blood cells) and the injectable product are great. When an injection solution is hypoosmotic, it contains fewer solute particles than the tissue fluid where it is injected; therefore, when injected into the muscle, the solution would cause tissue fluid to move away from the injection site/depot, resulting in an increase in the rate of passive diffusion. The converse is true for hyperosmotic pharmaceutical preparations.

(f) Volume of injection. As described above, for the unidirectional flow of a drug given by Fick's equation, when the volume of a pharmaceutical solution decreases, the diffusion rate should, in theory, increase. An increase in the injection volume within a relatively confined area (such as muscle or subcutaneous tissue) results in the lowering of the tissue surface area-to-volume ratio, decreasing the passive diffusion (since it is proportional to surface area).

(g) Physical type of dosage form. The type of dosage form also affects the rate of drug release and absorption of the active drug product. For example, aqueous solutions have a much faster release profile than aqueous suspensions, which are faster than an emulsion (Aulton, 2002).

Physiologic Factors

There are many physiologic influences that have a profound influence on the absorption of an injectable drug product. Blood volume, capillary hydrostatic pressure, and osmotic pressure all play a role in the ability of a drug to ultimately reach its target site. In addition, body temperature, patient age, and the disease state of tissue have been shown to modify the kinetics of drug absorption.

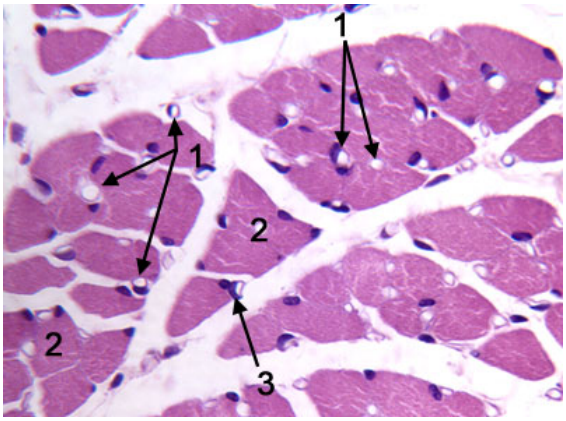
Furthermore, different anatomical sites are supplied with differences in the vascular netwrok, resulting in blood flow changes that may influence drug absorption. The quantitative blood supply to various organs and tissues of the body vary according to their functional requirements. For example, Binder *et al*. found that the intramuscular absorption of insulin was correlated to regional blood flow in the same individual (Binder *et al*., 1969). Because muscle can require up to 10 times the amount of blood during exercise, blood vessels supplying skeletal muscle are richly supplied with capillaries. It is for this reason that the rate of intramuscular absorption of some drugs closely approximates that of even intravenous administration.

Anatomical Factors

Factors unique to muscle tissue. Muscle tissue provides a drug transport environment in which dynamic muscle mechanical motion and loading through contraction (from, for example, exercise) may be a predominant influence on the systemic exposure of locally delivered agents. These motions and loads, which are shaped by both structure and function of the muscle, can present significant and variable physical influences on aqueous drug transport by means of their effect on modulating the extracellular space or fluid distribution (Sreter, 1963).

The continuous network of force-transmitting and connective perimysial and endomysial collagen fibers between myofibers can hinder interstitial diffusion in a strain-dependent manner. Dynamic physical effects such as intramuscular pressure, fluid redistribution, and structural deformations during mechanical function can also alter transport kinetics (Gajdosik, 2001). Cyclic strain may modify the accessible space, interstitial permeability, and transport kinetics for drug absorption intramuscularly. When the cyclic strain of muscles causes the drug to be exposed to larger anatomical volumes for absorption, spatial and temporal concentration gradients may result that increase the diffusional driving force. This can be caused by myofiber deformation, displacement, and thinning due to conservation of volume during elongation that increase tissue porosity. Interstitial permeability may increase due to alignment of collagen fibers in their dense interstitial networks that form the endomysium and perimysium during stretch, which reduces the permeability of a soluble drug (Purslow, 1989). Such changes may result in a greater time-averaged porosity and permeability that increases penetration.

Whereas local intramuscular delivery minimizes systemic losses and enables efficient administration of drugs to target tissues, it is clear that ultimate drug distribution and pharmacologic effects are, in large part, determined by target tissue pharmacokinetics, physiology, anatomy, and mechanical influences (Wu and Edelman, 2008). Transvascular transport of pharmaceuticals usually occurs at the level of continuous capillaries that are intermingled among the skeletal muscle tissue (Figure 1) (Becker, Woodley, & Baxter, 2009; Netter, 2006).



1. Capillary in cross section
2. Skeletal muscle like that which would be found in the Vastus Lateralis Region
3. Endothelial cell nuclei

Figure 1: Skeletal Muscle Tissue

Following absorption into the capillaries (Figure 2), the pharmaceutical product then moves into venules, then into the veins that reach the central circulation.

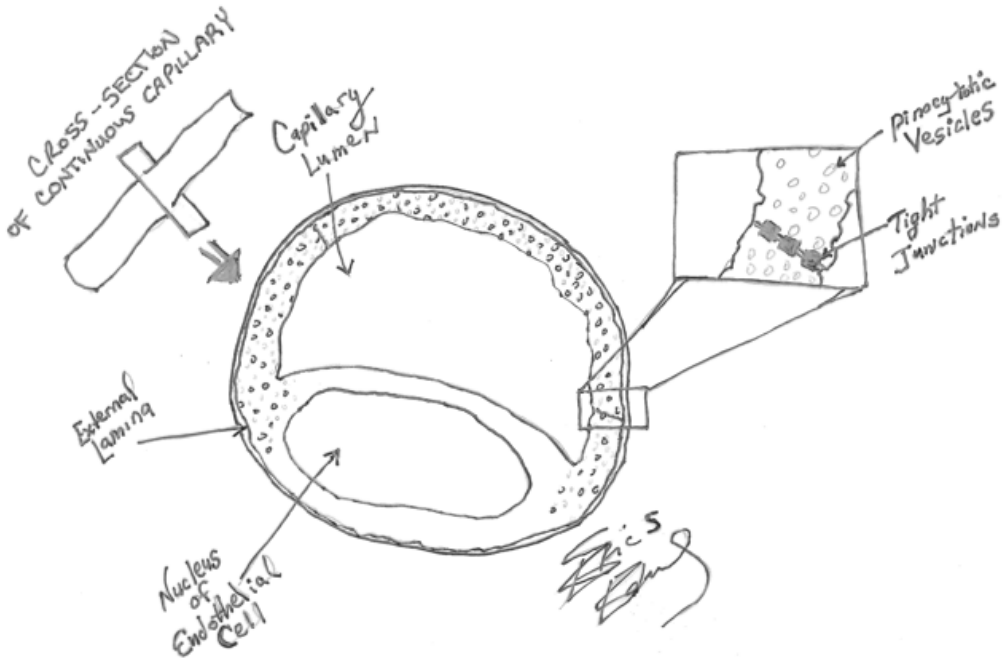


Figure 2: Cross-Section of Typical Continuous Capillary

Factors unique to subcutaneous tissue. Subcutaneous injections pierce the epidermal and dermal layers of the skin and deliver the drug into the loose subcutaneous tissue that includes adipocytes (fat cells) (Figure 3). Such injectable products are typically prepared as aqueous solutions or as suspensions. Following injection, drugs enter the capillaries or lymphatic system from the interstitial spaces by diffusion or filtration (Ansel, Allen, & Popovich, 2004).

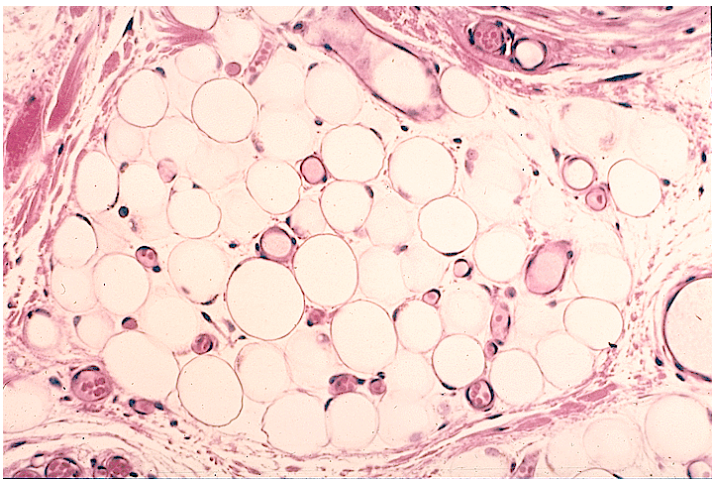


Figure 3: Adipose Tissue in the Subcutaneous Compartment

The structural characteristics in the interstitial spaces of the subcutaneous compartment are similar to the muscle compartment, consisting of a fibrous collagen framework (Figure 4) supporting a gel phase made up of glycosaminoglycans, salts, and plasma-derived proteins. The glycosaminoglycans are polyanionic polysaccharides that are fully charged at physiological pH and are bound covalently to a protein backbone to form proteoglycans which are immobilized in the interstitium, with the exception of hyaluronan. Hyaluronan is not immobilized and may be removed from the interstitium via the lymph in a flow-dependent manner (Lebel, Smith, Risberg, Gerdin, & Laurent, 1988; Lebel, Smith, Risberg, Laurent, & Gerdin, 1989; Pou, Roselli, Parker, & Clanton, 1993).

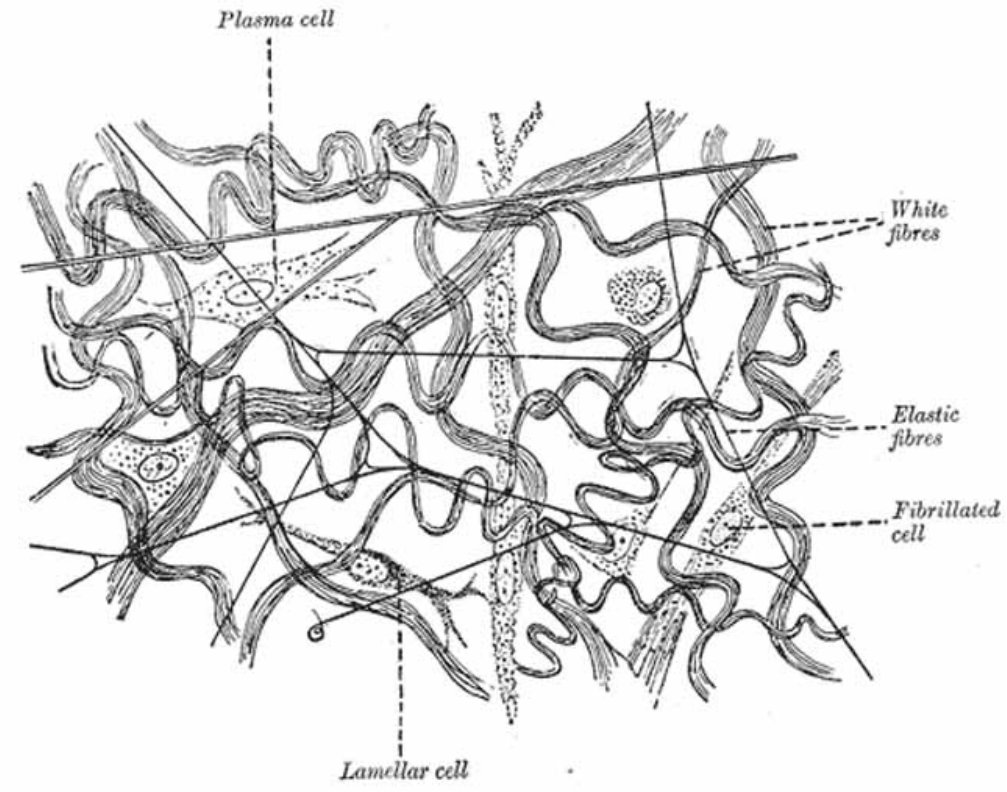


Figure 4: Subcutaneous Tissue Matrix Components

Absorption of drugs from the subcutaneous tissue is influenced, in large part, by the same factors that determine the rate and extent of absorption from intramuscular sites; however, the vascularity of subcutaneous tissue is less than that of muscle tissue and the additional extracellular matrix components of the interstitial architecture pose unique challenges to the administration of drugs subcutaneously. For example, decreased blood flow in the subcutaneous tissue, especially in diseased states, may lead to slower absorption as compared to intramuscular administration (Wilkinson, 2001). Older age may also lead to changes in absorption of drugs from the subcutaneous tissue compartment. One example of this includes the fact that older individuals are prone to hypothermia which may lead to SC vasoconstriction earlier and to a

greater extent as compared to muscle tissue (Inouye, 2004). Furthermore, diffusion of macromolecules within the interstitium may be physically retarded by the fibrous collagen network and the gel structure of the proteoglycans as well as by electrostatic interaction with charged components of the interstitial architecture (Porter & Charman, 2000).

Another substantial difference that characterizes the subcutaneous tissue compartment as compared to the intramuscular compartment is the availability of the lymphatic system to aid in the absorption of drugs. The blood capillaries supplying the subcutaneous tissue are generally continuous and are characterized by tight interendothelial junctions and an uninterrupted basement membrane. These blood capillaries are relatively permeable to the exchange of small, lipophilic molecules, and by virtue of capillary pores, some hydrophilic compounds. In contrast, the endothelium of blood vessels constitutes a significant barrier to the transfer of large, hydrophilic molecules such as proteins. Since the capillary endothelial barrier is relatively poorly permeable to large hydrophilic macromolecules, many proteins may be primarily cleared from the interstitium via the lymph. It is still unknown what causes the driving force for pharmaceutical transfer from the interstitium to the initial lymphatics, but some authors postulate it may be due to the potential energy difference in the fluid phase between the interstitium and lymphatics taking the form of a chemical gradient, with associated osmotic or oncotic pressure gradients, or a hydrostatic pressure differential (Casley-Smith Jr., 1982).

Mechanical Administration Factors

Achieving the desired clinical outcome following administration by injection depends on several mechanical administration factors. This includes utilizing a consistent administration technique, choosing the correct needle length and gauge for needle-based systems, ensuring that

the fluid jet penetrates into the correct tissue compartment (for needleless systems), and understanding the kinematics of injection that may be introduced by various injectable drug delivery technologies.

Studies have demonstrated substantial variability in techniques of administration for drugs injected subcutaneously or intramuscularly. For example, Katsma and Smith (1997) studied syringe and needle motion during simulated intramuscular injections into a skin pad model by 30 novices (students) and 29 experienced healthcare providers (nurses). The kinematics of injection, including the variables of vertical and horizontal needle displacement, depth of injection, peak and contact velocity, angle at skin contact, path of injection width, and angle at completion of injection were assessed using video motion analysis (Figure 5).

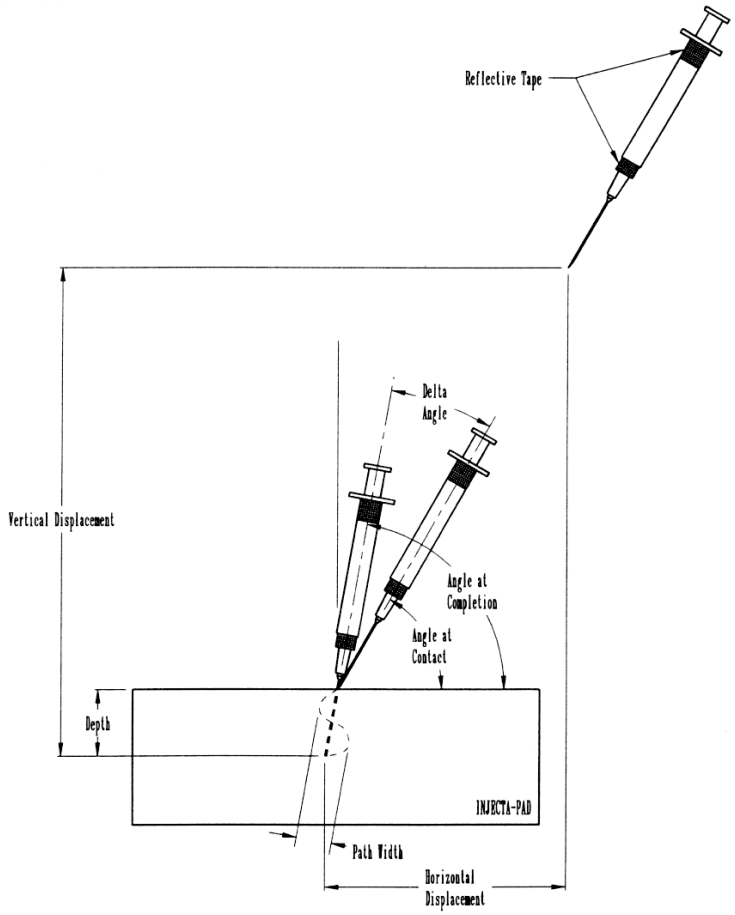


Figure 5: Analysis of Injection Kinematics

The results demonstrated substantial differences in several of the aforementioned variables, including vertical needle displacement, peak velocity, path of injection width, and angle at skin contact within and between the two groups that would have led to patients receiving subcutaneous versus intramuscular injections in practice (Katsma & Smith, 1997). The findings between the two groups are important in light of the fact that many injectable drugs are administered by novices in the out-of-hospital setting versus being historically administered by trained healthcare providers in a hospital or other medical setting.

One potential reason for the mechanical administration variability is the lack of standardized training. For example, healthcare professionals throughout medical professional schools in America are taught two different ways of administering IM injections. The first, widely used in the United States, requires bunching the thigh muscle at the injection site to increase muscle mass and to minimize the chance of striking bone (Bergeson, Singer, & Kaplan, 1982). The second, recommended by the World Health Organization (WHO), suggests stretching the skin flat between the finger and thumb, and pushing the needle down at a 90 degree angle through the skin (World Health Organization, 1984). Furthermore, there are limited opportunities for both student and registered nurses to perform injections in practice, and as such, knowledge and skills deteriorate over time (Hemsworth, 2000). Finally, existing practice variability may be compounded by new delivery technologies, such as pre-filled syringe devices, pen, or auto-injector systems that may require new techniques. Indeed, a literature review in the *Nursing Times* perhaps provides the best summary of the challenges regarding proper injection technique training by stating "few articles appear to re-examine the evidence for intramuscular injection technique; they often repeat opinions and anecdotes with little supporting evidence. A literature review of Medline, Cinahl and Cochrane databases found little evidence on injection theory and no evidence for aspiration of the syringe plunger. Studies have been undertaken on steps such as site selection and needle depth but have not always been rigorous comparative studies; this indicates the need for further research" (Malkin, 2008).

With the advent of a variety of injectable delivery technologies, a keen understanding of the physics of injection must be understood as a part of any drug/device development program. Schramm-Baxter and colleagues assessed needle-free jet injection dynamics into human skin

utilizing a commercially available, spring-driven injector system, Vitajet 3 (Bioject Inc.; Portland, OR). Further, injection kinematics for a needle-free system was provided by a derivation of Poiseuille's law:

$$P_0 = \frac{1}{8}\pi\rho D_0 \mu_0 \quad \text{where} \quad \mu_{average} = \frac{Q}{A_n t}$$

and

P_0 = power of the fluid jet

ρ = density of the Injectable

D_0 = exit diameter

μ_0 = exit velocity

A_n = cross-Sectional Area of the Needle

t = duration of Injection

Q = amount of Fluid Injection

The authors summarize that the power of an injection fluid stream and resultant penetration depth and associated dispersion into the tissue depends mainly on exit diameter (the diameter of the needle or needle-free orifice) and injection velocity. This equation may be applied to any delivery technology with some substantial force acting on a piston to expel an injectable medicament through an orifice (whether from a needle with lumen of "*x*" diameter or nozzle in a needle-free jet injector), as is the case with many pen and auto-injector delivery systems currently available or in development. The application of physics, including Poiseuille's equation, to an understanding of absorption dynamics affecting bioavailability that may be introduced by spring, gas or other "powered" delivery systems warrants further investigation that is beyond the scope of this research project.

Delivery System Factors

Previous studies have demonstrated differences in the bioavailability of an injectable drug product between different pharmaceutical delivery technologies (Bennett, Nichols, Rosenblum, & Condry, 1998; Brearly, Priestley, Leighton-Scott, & Christen, 2007; Simons, Gu, Simons, 2001). For example, in the study by Bennett and colleagues that investigated midazolam administered by a conventional syringe and needle compared to a jet injection system, it was found that the jet injector reached peak midazolam plasma concentrations over 30% faster with a significantly greater overall peak level (Bennett, *et al*., 1998). Similar studies with insulin have demonstrated substantial pharmacokinetic differences between different delivery systems (Kerum, Profozic, Granic, & Skrabalo, 1987; Halle, Lambert, Lindmayer, Menassa, Coutu, Moghrabi, Legendre, Legault, & Lalumiere, 1986; Taylor, Home, & Alberti, 1981).

More importantly, there have been reports of adverse or sub-optimal outcomes as a result of choosing the wrong delivery system for the desired clinical response. One example of this may be seen with the drug peramivir which was being developed for seasonal flu by Pharmaceuticals, Inc. where in a 2007 press release they stated "peramivir for seasonal flu failed to meet the primary endpoint in the mid-stage trial because too-short needles failed to deliver the drug to the muscle in all of the patients (Biocryst, 2007). As a result of this release, shares of the company fell more than 30% providing an example of the economic implications that can arise from a company not fully appreciating the potential impact of injection dynamics prior to conducting a large clinical trial to support drug development.

Models to Assess the Dispersion of Drug Administered by Intramuscular or Subcutaneous Routes

Introduction

Although injectable drug delivery is one of the most common administration routes, there is little knowledge about what happens to formulations following injection into the tissue. As previously discussed, pharmaceutical companies have conventionally focused on developing injectable products without consideration for dynamics that may introduce pharmacotherapeutic variability at the point the drug is introduced into the target tissue. Recognizing the need for direction on assessing these and other variables, the FDA recently issued a draft Guidance for Industry entitled "Technical Considerations for Pen, Jet, and Related Injectors Intended for Use with Drugs and Biological Products." Within this guidance, there is a subsection of Performance Testing: Injector and Drug/Biological Product Considerations that discusses assessment of depth and route of injections. The sections states, "testing should demonstrate that the depth of needle penetration and/or dispersion of the injectate are consistent. The model chosen for testing should simulate human skin and any specific tissue layers as closely as possible. The application should include an explanation to justify the model chosen for testing" (Food and Drug Administration, 2009). In order to understand models that have been previously developed to investigate injectable pharmaceutical dispersion in accordance with this guidance, a literature review was conducted.

Methods

Pubmed (Medline), ISI Web of Science, SCIRUS and Google Scholar were screened by title and abstract to identify literature for potential relevance. This search was originally

conducted in 2008 and was updated in September 2011. Keywords searched included injectable dispersion, intramuscular dispersion, intramuscular injection dispersion, subcutaneous dispersion, intramuscular injection deposition, injection absorption dynamics, intramuscular visualization, and subcutaneous visualization. The complete text was retrieved, and using specific inclusion and exclusion criteria, as described in Figure 6, articles were selected for consideration.

Results

Article selection. Table 1 provides a summary of studies meeting the aforementioned search criteria characterized by technique utilized to assess injection dynamics, the route of

Table 1: Summary of Literature Review Results for Models to Assess Dynamics Associated with Injectable Drug Delivery

Author	Technique	Injection	*In-Vitro* or *In-Vivo*	Species/ Model	Delivery system
Cash CJ, *et al*.	Ultrasound	ID and SQ	Both	Porcine & Human (n=1)	Needle-free injector
Donnelly, *et al*.	Radiolabeled ALA & Dye	ID	*In-vitro*	Porcine skin & Hydrogel	SQ-Pen
Madhu B., *et al*.	MRI	SQ	*In-vivo*	Porcine & Rat	Needle & Syringe
Schramm-Baxter, *et al*.	Radiolabeled mannitol & Dye	ID	*In-vitro*	Cadaveric	Vitajet 3 (needleless)
Wagner S., *et al*.	Fluorescent Dye	SQ	*In-vitro*	Porcine	INJEX
Fishman S., *et al*.	Fluoroscopy/ Electro-myographic Guidance	IM	*In-vivo*	Human	Needle & Syringe

ID = intradermal, SQ = subcutaneous, IM = intramuscular

administration, whether the study was *in-vitro* or *in-vivo*, the species or model chosen, and the delivery system utilized.

Thirteen articles met the inclusion/exclusion criteria, of which, six provide unique approaches to assess the dispersion of injectables (Figure 6) (Cash, Berman, Treece, Gee, & Prager, 2004; Donnelly, Morrow, McCarron, Garland, & Woolfson, 2007; Fishman, Caneris, Bandman, Audette, Borsook, 1998; Madhu, Elmroth, Lundgren, Abrahamsson, & Soussi, 2002; Schramm-Baxter & Mitragotri; Wagner, Dues, Sawitzky, Frey, & Christ, 2004).

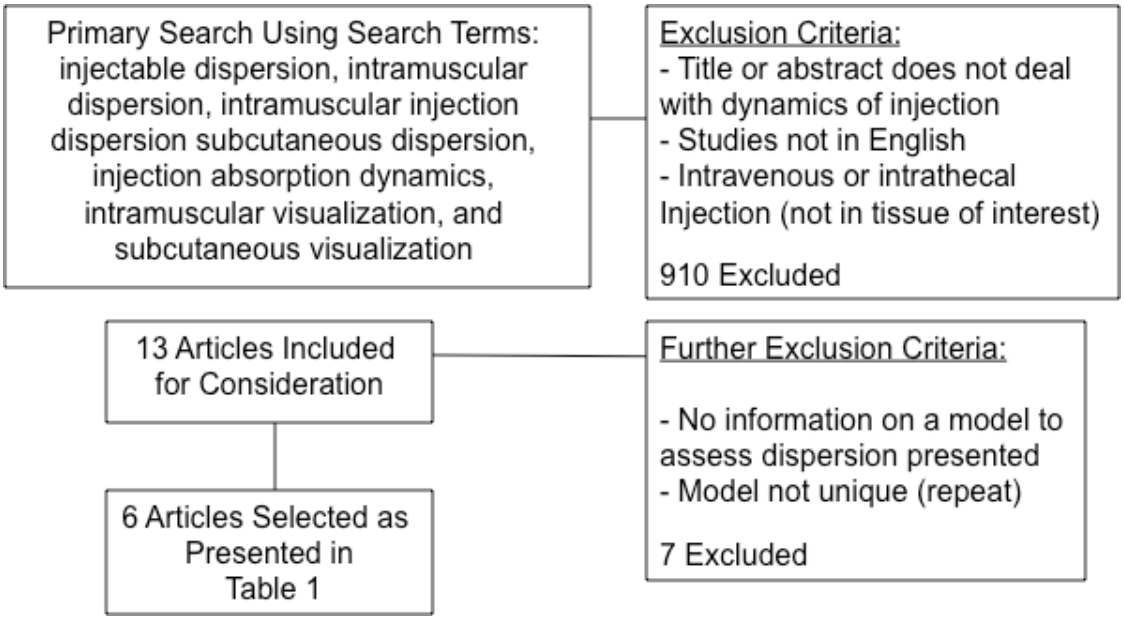


Figure 6: Literature Search Methodology

Analysis. The majority of the studies utilized *in-vitro* approaches to assess injections administered intradermally or subcutaneously. Models included injections into gels (polyacrylamide or hydrogel), animal models (e.g., porcine) or cadaveric skin/tissue using fluoroscopy, MRI, ultrasound, radiolabeled mannitol, or colorimetric dyes to examine the fate of the injectate (Bremseth & Pass, 2001; Bennett, Mundell, & Monheim, 1971; Brujan, Nahen, Schmidt, & Vogel, 2001; Fishman, *et al*., 1998; Madhu, *et al*., 2002; Schramm-Baxter, *et al*., 2004a; Schramm-Baxter, & Mitragotri, 2002; Schramm-Baxter & Mitragotri, 2004b; Smith, Hurdle, Locketz, & Wisniewski, 2006; Thow, Coulthard, & Home, 1992; Wagner, *et al*. 2004; Wendell, Hemond, Hogan, Taberner, & Hunter, 2006; Cash, *et al*. 2004). Most models were developed to assess the dynamics of jet injectors versus needle-based systems. Donnelly *et al*. studied the influence of solution viscosity and injection protocol on distribution patterns of jet injection with its application to photodynamic therapy targeting deep or nodular skin tumors. For this investigation they utilized two models. First, a hydrogel was developed by cross-linking sodium tetraborate decahydrate with hydroxyl groups on polyvinyl alcohol (PVA) molecules. Following mixing, the PVA-borate was then heated for complete homogeneous gel formation at 80 degrees C for 2 hours, then cooled at room temperature for 48 hours. Injections used methylene blue with PVA prepared at different viscosities. For the next model, the investigators used full thickness neonate porcine skin from stillborn piglets that were injected with a PVA solution containing a photosensitizer, tripropyleneglycol monomethyl ether (TMP). A factorial experimental design was then employed such that the effect of five viscosities, three injection volumes, and three standoff distances could be simultaneously evaluated. The dispersion pattern was quantified using a digital micrometer assessing the total depth of jet penetration (*Lt*), the

maximum width of the penetration pattern formed (*Lw*), and the depth at which the maximum width occurred (*Lm*) (Figure 7) (Donnelly, *et al.*, 2007).

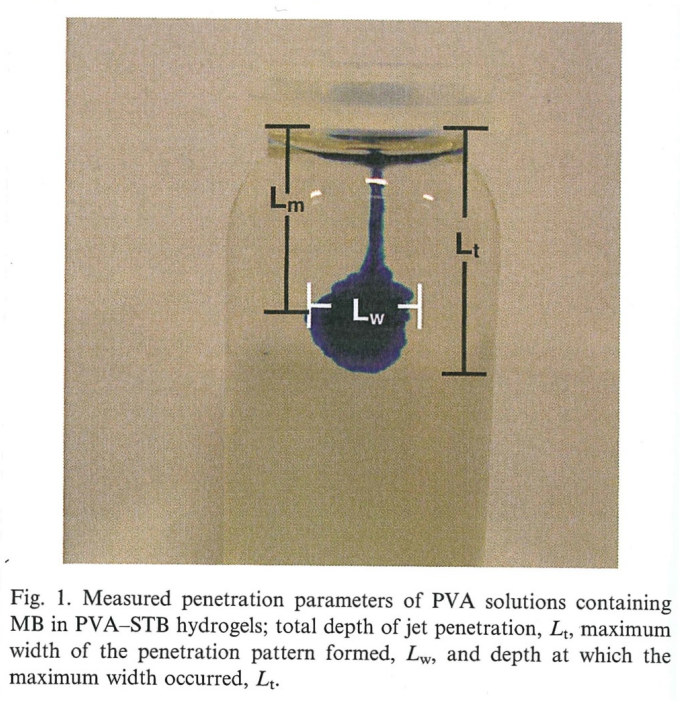


Fig. 1. Measured penetration parameters of PVA solutions containing MB in PVA–STB hydrogels; total depth of jet penetration, L_t, maximum width of the penetration pattern formed, L_w, and depth at which the maximum width occurred, L_t.

Figure 7: Measured Penetration Parameters of PVA Solutions containing MB in PVA-STB Hydrogels.

A result of the dispersion in porcine skin may be found in Figure 8 below. The investigators were unable to correlate the hydrogel findings with the porcine model for any of the parameters studied.

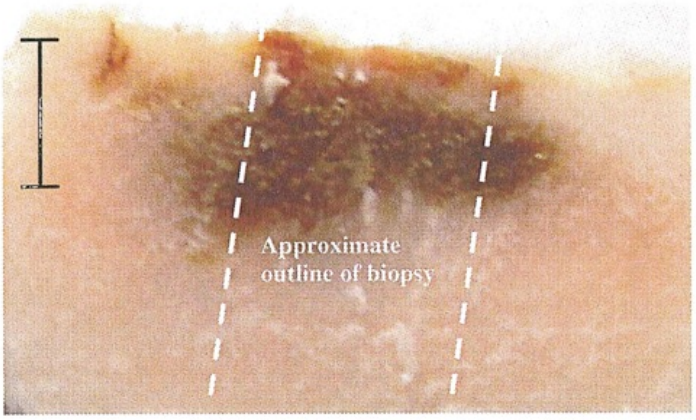


Figure 8: Dispersion in Porcine Skin

In another study, human dorsal abdominal skin was procured through the National Disease Research Interchange and was frozen at −70 °C until the time of experiments. Jet penetration into human skin was quantified using radiolabeled mannitol and jet dispersion was assessed using a colorimetric dye, sulforhodamine B (SRB). The authors noted, "the experimental setup was previously validated to represent *in-vivo* jet injections." However, the "*in-vivo*" correlation for this study was done in a porcine model instead of a human model. The authors of this study validated the concern noting, "porcine skin is a good model of human skin for testing diffusive permeability; however, this similarity may not extend to jet delivery, where the mechanical properties of skin might be important" (Schramm-Baxter & Mitragotri, 2004a).

Only one study could be located that attempted to correlate dispersion of an injectable with the bioavailability of the drug. This study was located following the formal literature review as described above and included an animal model that measured intradermal fluid conductivity by infusing mice with human serum albumin with or without the co-administration of an

investigational enzyme, recombinant human hyaluronidase. This recombinant hyaluronidase is being developed as a "spreading agent" to enhance the drug delivery of local anesthesia, contrast agents, and for subcutaneous fluid replacement (also called hypodermoclysis). The model assessed the dispersion characteristics and pharmacokinetics of anti-TNF-alpha antibody and PEGylated interferon.

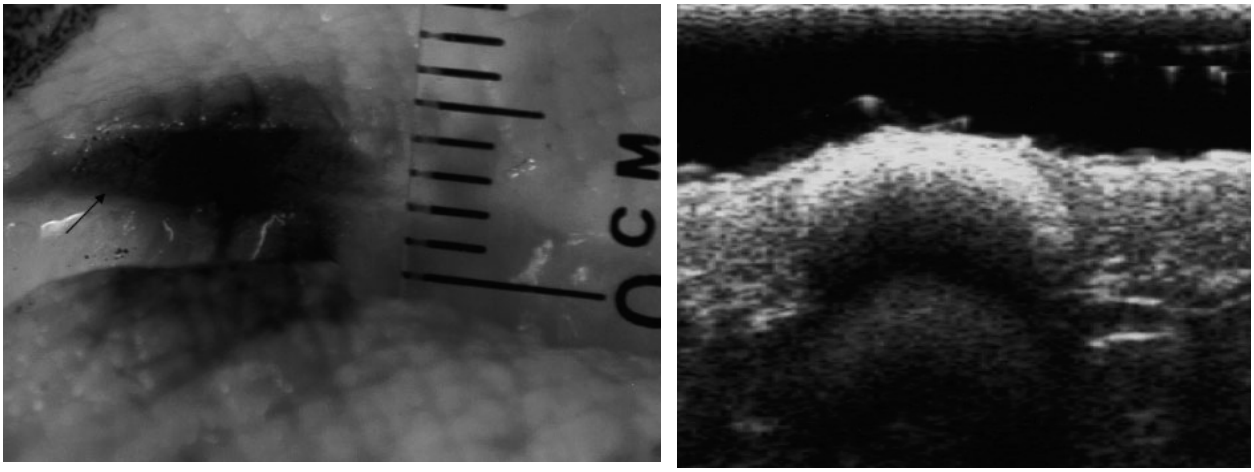
Flow rates were determined by measuring the time required to inject a known volume of the substance. Intradermal drug dispersion was assessed by co-injecting fluoresceinated dextran and the hyaluronidase at various doses. Images of the injection site were recorded and the area of dispersion was quantified using the fluorescent signal as an indicator. Pharmacokinetic parameters of radiolabeled biotherapeutics alone (anti-TNF-alpha antibody and PEGylated interferon) or co-administered with the hyaluronidase were analyzed following intravenous and intradermal injection into the rats. The reversibility of the effects of hyaluronidase was assessed by injecting hyaluronidase intradermally followed by trypan blue dye injection at the same site from 0.5 to 48 hours later and quantifying the area of dispersion.

The researchers found that drugs co-injected with hyaluronidase intradermally were more effectively dispersed, with up to a 20-fold increase in hydraulic conductivity ($p<0.05$) and reduced tissue distortion at the injection site as compared to the drugs injected without the hyaluronidase. This was accompanied by a significantly increased systemic bioavailability of co-administered biotherapeutics (116% vs. 64%, p=0.002, for PEGylated interferon) and faster time to maximal blood concentration (Tmax of 8 hours vs. 24 hours, for anti-TNF-alpha monoclonal antibody), demonstrating pharmacokinetics approaching those obtained by intravenous dosing of the biotherapeutics (Haller, Bookbinder, Keller, Hofer, Radi, Lim, & Frost, 2006).

Authors in several studies note that the opaqueness and composite structure of the skin makes it difficult or nearly impossible to visualize dispersion within or below the skin in real time (Donnelly, *et al*., 2007; Schramm-Baxter, Katrenick, & Mitragotri, 2004; Schramm-Baxter & Mitragotri, 2004). Although the development of an approach that would be able to characterize dispersion in real-time seems difficult at best, a component could include a radiographic imaging technique that allows for direct visualization of a radiolabeled injectate or imaging agent. This agent would also allow for characterization of systemic exposure measurements following administration over time using traditional bioanalytical techniques.

Two studies were located that utilized imaging techniques to characterize profiles of an injectate, one using ultrasound technology and the other with magnetic resonance imaging (MRI) (Cash, *et al*., 2004; Madhu, *et al*., 2002). This is surprising given the fact that radioimaging technologies have been incorporated into pharmaceutical research of pulmonary delivered systems for years (Dolovich, 2001; Newman, Pitcairn, Hirst, & Rankin, 2003; Fleming & Conway JH, 2001; Hasani, Agnew, & Toms, *et al*., 1999; Fleming, Quint, & Bolt, *et al*., 2006). The study by Cash and colleagues incorporated two- and three-dimensional ultrasound in the development of a needle-free injection system in order to differentiate and quantify the amount of injectate that arrives into the dermis or subcutaneous tissue. For this study, the researchers conducted two studies, one *in-vitro* and another *in-vivo*. The *in-vitro* approach used fresh pig loin for a serious of injections with a jet injection device (Weston Medical jet injector). The loin was scanned using a Daisus ultrasound machine at 16 and 22 megahertz (Mhz) frequencies, confirming the injectate location with electronic calipers and dissection. They then injected 0.15mL and 0.5mL of the injectate into the abdominal wall of humans where 2-D images were

converted to 3-D images using processing software that allowed them to assess the position of the injectate in relation to the layer of skin penetrated. *In-vitro*, investigators were able to differentiate between the dermis and SQ tissues with the ultrasound device casting a strong acoustic shadow that showed an arc diameter clearly beneath the dermis. Upon dissection, injectate location was verified. However, the *in-vivo* experiment was unable to demonstrate a clear injection beneath the dermis (Figure 9).



Left: *in-vitro* intradermal pig loin injection dissection showing injectate into dermis
Right: ultrasound following *in-vivo* intradermal human abdomen injection

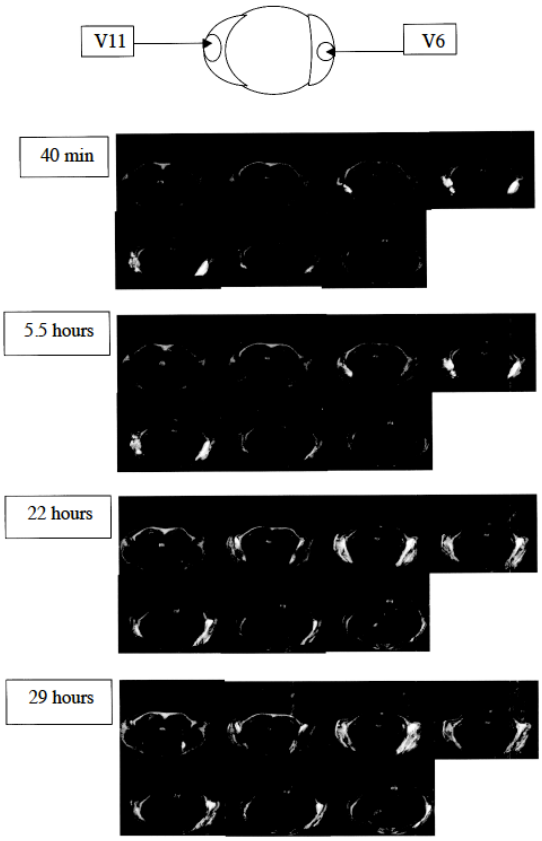
Figure 9: Intradermal Injections in Pig Loin (Left) and Human Abdomen (Right)

The strengths of this study include the fact the ultrasound technology is non-invasive and easy to use in studying a variety of injectable techniques and delivery systems. Weaknesses include the lack of information provided on the injectate used, number of subjects that could provide a measure of reproducibility, and the *in-vivo* results not being able to clearly define the location of the injectate. Thus, the practical applicability of this method to researchers or industry may be limited (Cash, *et al*., 2004).

Madhu and colleagues investigated the use of MRI for studying various subcutaneous formulations *in-vivo* without the use of typical marker substances or contrast enhancing agents that are normally associated with MRI research. Vehicles assessed included a variety of formulations typically incorporated into controlled release pharmaceutical products including oils, a lipid emulsion, water solutions of cyclodextrin, normal saline, and block co-polymers (poloxamers) that were visualized *in-vitro* (in a beaker) and *in-vivo* (pig flesh and the fatty chest wall of rats) by a ^{1}H-MRI technique. Poloxamers are surfactants that can be used to increase the water solubility of hydrophobic, oily substances or otherwise increase the miscibility of two substances with different hydrophobicities, hence their use in controlled release pharmaceutical preparations. The *in-vitro* studies were conducted to assess visibility prior to the *in-vivo* experiments by injecting 0.5mL of each vehicle into a beaker followed by MRI scanning. Next, 0.5ml of each vehicle was injected into the SQ tissue of pig flesh. Finally, 0.5ml of select vehicles were injected into the SQ region of the chest wall (due to its thick distribution) and rats were placed-belly down into the scanner and assessed over several time points (up to over 20 hours). The pattern area of the vehicle was demarcated and measured through imaging software and the area was then multiplied by the MRI slice thickness to obtain the volume of the vehicle. Volumes were added to determine the total volume of injectate per unit time.

The authors found significant variability between what could be detected *in-vitro* as compared to the injections in the pig flesh and rat chest wall. The only vehicle that demonstrated consistent qualitative and quantitative detectability among all of the models were the block co-polymers. Specifically, Poloxamer 407 dissolved in water for injection were clearly present in the beaker and pig flesh and displayed the highest contrast in the rat chest wall (Figure 10). As

depicted in the below figure volume expansion occurred from the original injection, likely due to osmotic properties of the drug. In other words, expansion may have been a function of the higher osmotic pressure and diffusion rate of the vehicle.



V6 = Poloxamer 407 and 188 (18 and 10% respectively) dissolved in water for injection
V11 = Poloxamer 407 (16%) dissolved in water for injection

Figure 10: Poloxamer Detectability in Rat Chest Wall following MRI Scanning

The strengths of this MRI approach includes the ability to measure extended-release pharmaceutical preparations *in-vitro* first to assess detectability, followed by *in-vivo* confirmation. Other parameters such as formulation concentration, different volumes of injection, and different injection site locations may be further studied using this technique.

However, because block co-polymers are typically used with extended release pharmaceutical preparations coupled with the inability of MRI to distinguish the water based vehicles *in-vivo* in this study, applicability of this approach to conventional, non-controlled release products may also be limited (Madhu, B., *et al*., 2002).

Conclusions

Although some approaches have been used to assess dispersion *in-vitro*, there is a dearth of research that has been conducted to correlate any *in-vitro* approach with what would be expected to occur *in-vivo* in humans. Additionally, there are few approaches that have been suggested to investigate the dispersion of pharmaceuticals using available radioimaging technology and, of those reported, the practical application to pharmaceutical research using injectable delivery systems remains to be explored. Some of the *in-vitro* approaches presented provide useful information for the development of techniques to assess dispersion *in-vivo* using radiographic imaging technology. For example, the same measurements that were conducted *in-vitro* in hydrogels could be applied *in-vivo* using such measurement parameters as the total depth of injection penetration, the maximum width of the penetration pattern formed, the depth at which the maximum width occurs.

There is a significant need for new approaches to assess the dispersion dynamics associated with non-intravenously administered injectable products. These dynamics may have an impact on the bioavailability and systemic exposure profiles of these drugs, and therefore, may ultimately impact safety and efficacy. An approach that allows for the qualitative and quantitative dispersion assessment of an injectable at the moment of tissue deposition coupled with an assessment of exposure metrics following systemic absorption could provide substantial

information to researchers when developing new injection technologies. Such an approach would also afford the ability to assess a variety of injection dynamics as presented earlier in this chapter. Ultimately, incorporating quantitative *in-vivo* measurements that characterize the release of an injectable depot from the extravascular compartment into the systemic compartment could be correlated with an *in-vitro* approach. This potential *in-vitro*, *in-vivo* correlation approach could shorten the development time for injectable pharmaceutical products, initially developed in a vial and syringe format to be administered by healthcare providers, that are subsequently transitioned into delivery technologies designed for consumer use by providing a surrogate for *in-vivo* bioavailability studies.

CHAPTER 3. PILOT STUDY TO ASSESS THE DISCRIMINATORY CAPACITY AND SAFETY OF COMPUTED TOMOGRAPHY SCANNING AS WELL AS THE USABILITY OF IOHEXOL AS AN INJECTABLE STANDARD FOR THE INVESTIGATION OF DISPERSION CHARACTERISTICS BY PARENTERAL ADMINISTRATION SYSTEMS

Background and Objectives

The primary objective of this pilot study was to evaluate the safety and usability of Computed Tomography (CT) scanning along with iohexol, a non-ionic, radiocontrast imaging injectable agent. This information provided the foundation for the development of an approach for assessing dynamics of dispersion that may be introduced by injectable delivery technologies.

Selection of Radiographic Imaging Approach

As reported in Chapter 2, there are few studies that discuss the assessment of pharmaceutical dispersion through the use of radiographic imaging technology. In developing an approach for assessing dispersion of an injectable using available imaging techniques, overall imaging requirements should be matched to the appropriate imaging technology. An imaging system should be able to assess, qualitatively and quantitatively, a wide variety of variables or dynamics associated with the *tissue* bioavailability of a drug following administration by different delivery technologies. Ideally, the imaging technique should allow researchers to

1) view/discriminate accurately where an injectable depot is located in the tissue compartment immediately following an injection and 2) subsequently, upon evaluation of the radiographic images, allow for the application of techniques to measure quantitative dispersion characteristics such as location of dispersion, depth of penetration, and width of penetration. Furthermore, the ability to detect changes in location and certain parameters over time would be important when trying to correlate tissue compartment elimination with the absorption of a pharmaceutical product into the systemic circulation. Other imaging requirements for assessing dispersion characteristics include the need for excellent resolution in multiple imaging planes as well as the ability to apply imaging settings to multiple research subjects. Finally, any approach, and its constituent imaging technology component, should be able to be validated against intended requirements. This includes at a minimum, that ability to test attributes of specificity, accuracy, precision, and reproducibility. Given that most radiographic imaging technologies were developed for clinical/medical use, any imaging technology should be relatively readily available for research and should not be cost prohibitive.

Studies that have incorporated traditional medical imaging approaches have preliminarily assessed MRI and ultrasound technology (Cash, *et al*., 2004; Madhu, *et al*., 2002). Although ultrasound technology can provide images in real time and is relatively inexpensive, the technology has been shown to be unable to provide clear resolution for an injectate administered below the dermis due to the inability to detect a focal acoustic impedance signal (Cash, *et al*., 2004). Magnetic resonance imaging has demonstrated promise as a noninvasive, non-ionizing radiation producing technology that can distinguish certain controlled release preparations in animal models and, through the use of marker substances such as gadolinium, could prove useful

for certain investigations of injectable delivery systems (Madhu, *et al*., 2002). However, MRI is costly and requires a substantial amount of time to acquire an image (usually over 20 minutes per scan) limiting the ability to investigate early dispersion characteristics.

One overlooked imaging approach that may meet the aforementioned requirements is modern multi-slice computed tomography scanning incorporating a non-ionic, radiocontrast agent as a model injectable. Computed tomography scanning has been available since the early seventies (Beckmann, 2006). This imaging technology incorporates the use of an x-ray source, imaging sensor and computer processing that generates cross-sectional slices, or tomograms, of a test component or anatomical structure. Pixels of each slice are displayed according to a measure of tissue attenuation that translates into relative radiodensity. Once a scan has been acquired, the radiodensity data must be processed using reconstruction software that produces a series of cross-sectional images for viewing on a monitor. CT scanning technology has evolved drastically over the last several decades moving from single technology to spiral CT in the early 90's allowing for the acquisition of imaging data without misregistering anatomical details, to multislice CT systems (MSCT) the can simultaneous acquire data from multiple slices per x-ray rotation (Crawford & King, 1990). This provides considerable improvement towards isotropic three-dimensional imaging. In fact, modern MSCT scanners can acquire 64 slices per rotation with an isotropic resolution in the submillimeter range. These latest generation of scanners are extremely fast, able to conduct a "whole body" MSCT scan with a 1,500 mm scan range in under 25 seconds (Kohl, 2005). Additionally, due to the fast acquisition time, tissue changes may be examined in rapid succession at any desired time point. Modern technology has also allowed for

the optimization and minimization of radiation exposure to a patient. The safety of computed tomography is discussed in greater detail below.

Radiographic contrast media are utilized clinically in CT scanning to improve the visibility of internal body structures in various imaging techniques. Commonly used agents include iodine due to its relatively positive safety profile and its water solubility. Iodine may be bound in an organic compound or ionic compound. Typically, organic compounds have fewer side effects as they do not dissociate into component molecules. The iodine concentration determines the radiopacity and subsequent ability to assess changes in tissue radiography on CT. Most non-ionic, radiocontrast media are injected intravascularly for angiographic or venographic imaging studies. However, iodinated media has been approved for imaging of almost every human organ and body cavity including enhancement of computed tomography images for the liver, pancreas, brain, spine, kidneys, pelvis, abdominal cavity, and retroperitoneal space. As iodine is regulated as a pharmaceutical, is manufactured as a sterile injectable, and shares many properties of other small volume parenterals, it may be an ideal agent to study as an injectable when developing an approach to assess dispersion characteristics from injectable delivery technologies. Iodine is considered to be a safe contrast agent. It has been used for many years without serious side effects. The most common side effect of iodine is a warm or "flushed" sensation during the actual injection of the iodine, followed sometimes by a metallic taste in the mouth that usually lasts for less than a minute. Iodinated contrast may lead to allergic reactions, including some severe; however, this is very rare and newer agents have been developed to reduce this risk. The safety of non-ionic contrast media is discussed in greater detail below.

Finally, the use of radiographic tomographic imaging techniques coupled to an imaging agent or radiotracer is not new in pharmaceutical research involving evaluating parameters associated with different delivery/inhaler technologies. For example, in the inhaled drug/device combination product development arena, imaging approaches that involve radiation including single-photon emission computed tomography (SPECT) with a gamma-emitting radioisotope have been used for years to investigate and quantify lung deposition parameters (Fleming & Conway, 2001). However, as previously presented, dynamics introduced by injectable delivery technologies have been underappreciated and have led to a dearth of imaging technology applications in the injectable development arena.

Safety

Computed tomography. The safety of computed tomography has recently been brought to the forefront of public attention through articles presenting the dangers of radiation exposure and associated increase in cancer risk (Gutherie, 2008; LaPook, 2009). The articles discuss the challenges with our healthcare system leading to "defensive medicine" and the ordering of multiple, unnecessary CT scans in as many as a third of all CT scans. In addition, many radiologic technicians are not adjusting the settings of newer scanners to allow for reduced radiation exposure. One study demonstrated radiation doses from the identical CT procedure varying up to 13-fold among patients at the same institution (Smith-Bindman, Lipson, & Marcus, *et al*., 2009).

The radiation dose for a particular study depends on multiple factors: volume scanned, patient build, number and type of scan sequences, and desired resolution and image quality. In addition, two CT scanning parameters that can be adjusted easily and that have a profound effect

on radiation dose are tube current modulation and adjusting the pitch (movement of the patient/subject table through the scanner), thus limiting exposure time. Given the ability of new multislice scanners to provide a narrow scan field with radiation optimization features, it was hypothesized that computed tomography scanning of a narrow area in an extremity could limit radiation exposure and result in an associated risk/benefit profile that would be acceptable for research purposes. Hence the desire to test this through a pilot cadaveric study.

Non-ionic radiocontrast media. Most of adverse effects from non-ionic radiocontrast media occur soon after administration and are usually self-limiting and of short duration. However, some adverse effects may be delayed and may be of a long-lasting nature. Most reactions are usually of mild to moderate severity, occurring in less than 5% of patients when administered in the vein. Serious, life-threatening and fatal reactions, mostly of cardiovascular origin, have been associated with the intravenous administration of iodine-containing contrast media. These are rare, occurring in less than <0.3% of administrations. The injection of contrast media is frequently associated with the sensation of warmth and pain, especially in peripheral angiography; pain and warmth are less frequent and less severe with certain contrast media products, such as iohexol compared to others. There are other side effects that are reported, but are also rare, usually occurring in less than 0.5% of patient administrations (Omnipaque®, 2008).

Although non-ionic contrast media is not specifically indicated for direct visualization of tissue by subcutaneous or intramuscular administration, the agent has been administered for such purposes in clinical settings off-label. In one study, conducted by Fishman and colleagues, eleven patients who had symptoms of gluteal pain radiating down the affected leg with one or more associated signs of piriformis muscle related irritation of the sciatic nerve, underwent an

injection procedure that involved a tri-iodinated agent, iopamidol (Isovue), administered for anatomic verification combined with fluoroscopy and electromyographic guidance (Fishman, *et al*., 1998). Of the 17 injections that were performed in 11 subjects, the injection of the non-ionic contrast agent was well tolerated with no adverse events reported. During procedures that require contrast media administration, it is not uncommon for the iodine to be displaced into the extravascular tissue, including muscle and subcutaneous tissue. This usually occurs iatrogenically by a healthcare provider puncturing through the vessel following catheter insertion for administration of the dye or through the use of power injectors set at levels that are too high for some fragile vessels. Several studies have demonstrated that extravasation of non-ionic contrast media into the subcutaneous or intramuscular tissue usually results in minimal clinical significance (Jacobs, Birnbaum, & Langlotz, 1998; Sistrom, Gay, & Peffley, 1991; Wang, Cohan, Ellis, Adusumill, & Dunnick, 2007). For example, in one study, of the 442 adults who experienced extravasation from non-ionic contrast media, 97.7% (432) had minimal or no adverse effects, nine had moderate adverse effects, and one had a severe complication (75 mL of contrast material extravasated into the hand, causing compartment syndrome). Only one moderate or severe complication in an adult resulted from an extravasation of less than 50 mL. Extravasated volumes ranged from 3 to 150 mL and symptoms usually consisted of swelling and/or pain. The authors concluded that extravasation of nonionic iodinated contrast medium results only rarely in moderate or severe adverse effects, and these usually occur only when large volumes of contrast medium are involved (Wang, *et al*., 2007).

Methods

Computed Tomography Scanning and Dosimetry

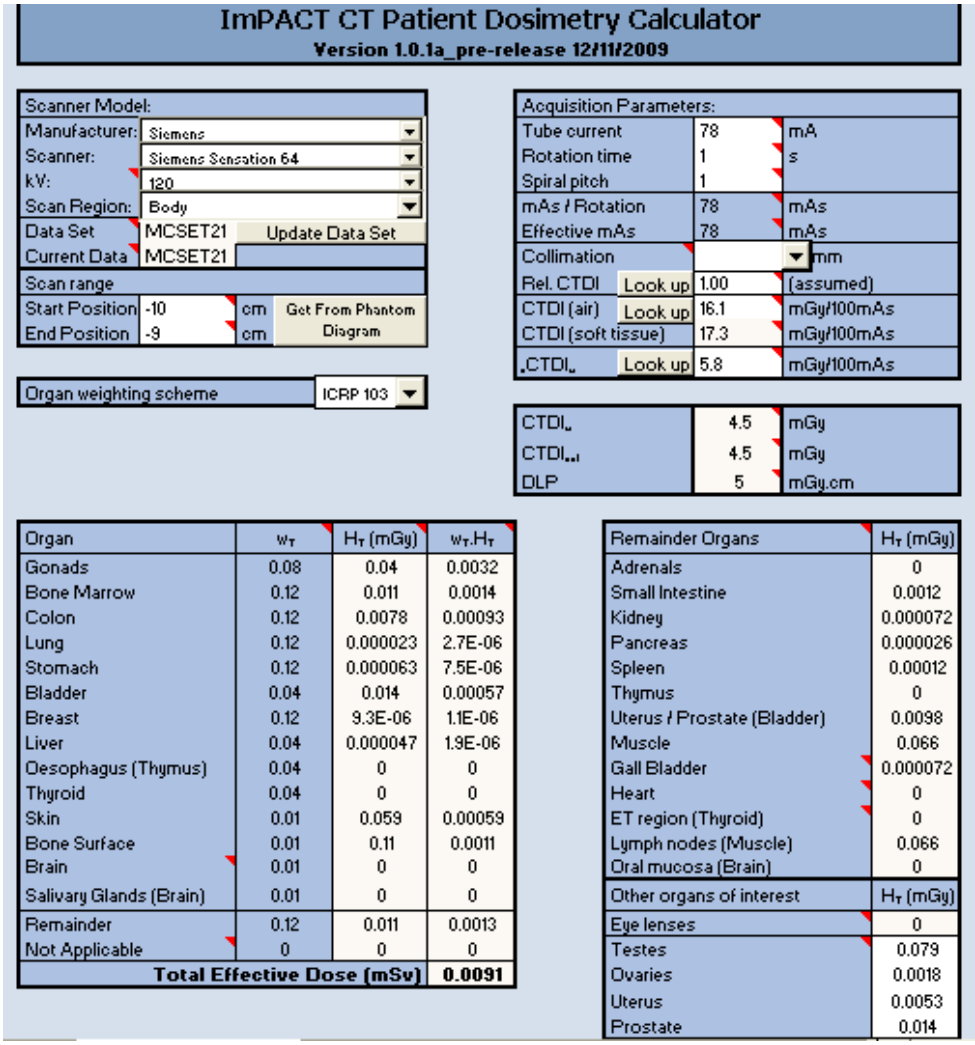
A cadaveric lower extremity was obtained, with permission, from the Virginia Commonwealth University (VCU) Department of Anatomy. The extremity was procured from a male human cadaver of approximately 68 years of age. CT scanning took place in the Radiology Department of the VCU Medical Center using the Somatom Sensation®, 64-slice CT scanner. The scanner provides high-speed, high-resolution sub-millimeter volume scanning (up to 87mm/s). An initial scan of the thigh region from approximately 3 inches below the pubis symphysis to approximately 2 inches above the patella (approximately 13 cm total scan length of the extremity) was conducted to localize the injection area as well as to determine the effective radiation dose human subjects would receive in the planned, follow-on *in-vivo* study. The scans were conducted by an experienced computed tomography radiology technician followed by dosimetry evaluation by two Radiological Physicists at the VCU Office of Radiation Safety utilizing the ImPACT CT dosimetry tool (ImPACT, London, UK).

Effective dose estimates the total amount of radiation absorbed by tissues, calculated as the weighted sum of the dose to irradiated organs and tissues (Payne, 2005). It is expressed in sievert (Sv) or millisievert (mSv) units (previously expressed as roentgen equivalent man (rem) units. Tissue weighting factors allow CT radiation doses to be calculated and adjusted in light of tissue-specific vulnerabilities, which minimizes the risks to subjects. Table 2 provides typical effective dose values for CT imaging examinations.

Table 2: Effective Dose Values for CT Imaging Examinations

Computed Tomography Examination	Typical Effective Dose (mSv)
Chest	5-7
Head	1-2
Abdomen and Pelvis	8-11
Coronary Artery Angiogram	9-12
Colon	6-11

The imPACT CT dosimetry tool includes software that incorporates complex Monte Carlo simulations to calculate effective dose involving the radiation beam, target scan volume, gantry motion and the tissue weighting factor values that reflect target organs' varying radiosensitivities. In other words, the calculated radiation dose delivered to each organ volume is multiplied by the relevant tissue weighting factors and the sum of these products is the effective dose. A sample screenshot from this software is located in Figure 11.



ImPACT CT Patient Dosimetry Calculator
Version 1.0.1a_pre-release 12/11/2009

Scanner Model:
Manufacturer: Siemens
Scanner: Siemens Sensation 64
kV: 120
Scan Region: Body
Data Set MCSET21 Update Data Set
Current Data MCSET21
Scan range
Start Position -10 cm Get From Phantom Diagram
End Position -9 cm
Organ weighting scheme ICRP 103

Acquisition Parameters:

Tube current	78	mA
Rotation time	1	s
Spiral pitch	1	
mAs / Rotation	78	mAs
Effective mAs	78	mAs
Collimation		mm
Rel. CTDI Look up	1.00	(assumed)
CTDI (air) Look up	16.1	mGy/100mAs
CTDI (soft tissue)	17.3	mGy/100mAs
CTDI Look up	5.8	mGy/100mAs

CTDI	4.5	mGy
CTDI	4.5	mGy
DLP	5	mGy.cm

Organ	w_T	H_T (mGy)	$w_T.H_T$
Gonads	0.08	0.04	0.0032
Bone Marrow	0.12	0.011	0.0014
Colon	0.12	0.0078	0.00093
Lung	0.12	0.000023	2.7E-06
Stomach	0.12	0.000063	7.5E-06
Bladder	0.04	0.014	0.00057
Breast	0.12	9.3E-06	1.1E-06
Liver	0.04	0.000047	1.9E-06
Oesophagus (Thymus)	0.04	0	0
Thyroid	0.04	0	0
Skin	0.01	0.059	0.00059
Bone Surface	0.01	0.11	0.0011
Brain	0.01	0	0
Salivary Glands (Brain)	0.01	0	0
Remainder	0.12	0.011	0.0013
Not Applicable	0	0	0
Total Effective Dose (mSv)			0.0091

Remainder Organs	H_T (mGy)
Adrenals	0
Small Intestine	0.0012
Kidney	0.000072
Pancreas	0.000026
Spleen	0.00012
Thymus	0
Uterus / Prostate (Bladder)	0.0098
Muscle	0.066
Gall Bladder	0.000072
Heart	0
ET region (Thyroid)	0
Lymph nodes (Muscle)	0.066
Oral mucosa (Brain)	0
Other organs of interest	**H_T (mGy)**
Eye lenses	0
Testes	0.079
Ovaries	0.0018
Uterus	0.0053
Prostate	0.014

Figure 11: ImPACT CT Sample Dosimetry Calculator

Radiocontrast Media (RCM) Injections and Image Acquisition

Four 1mL injections occurred in the vastus lateralis region of the cadaveric thigh using iohexol (a readily available, tri-iodinated, non-ionic, radiocontrast media) at a concentration of 300mgI/mL. Injections included the use of a 5/8" 25 gauge, subcutaneous needle attached to a 1 milliliter (mL) pre-filled syringe (PFS), and a 1" 23 gauge intramuscular (IM) needle attached to a PFS (2 injections). Images were then loaded into the Main VCU imaging database and

analyzed to determine the discriminatory capacity of the scanner to visualize the RCM, confirm the estimated radiation exposure, and verify that usable quantitative measurements could be achieved from this imaging approach.

Quantitative Analysis of Computed Tomography Images

Computed Tomography scans were processed utilizing iSite Picture Archiving and Communications System (iSite PACS, Philips Koninklijke Electronics, ver. 3.6.52) software. Images were enhanced using the Laplacian Method to increase the visibility of edges in each image slice. Measurements of maximum depth of dispersion (MDd) were completed utilizing a ruler tool in millimeter units by selecting the point on the skin surface (epidermal region) and measuring to the deepest detectable level of iohexol contrast media for a given axial image slice. Measurements of maximum width of dispersion (MWd) were completed utilizing the ruler tool by selecting the axial image slice with the widest tissue dispersion of iohexol. Finally, measurements of the depth at maximum width of dispersion (DMWd) were completed utilizing the ruler tool to measure the depth at the axial image slice with the widest tissue dispersion of iohexol at each time point by selecting the point on the skin surface (epidermal region) and measuring to the deepest detectable level of iohexol contrast media. These measurements were independently verified by a Radiologist to ensure inter-rater reliability of the measurements.

Results

Dosimetry

For this pilot study, critical CT scanner settings to maximize image resolution while minimizing radiation exposure were chosen through consultation with VCU radiologists and radiology physicists. These included selecting a tube-current of 70 milliamps (mA) and peak

kilovoltage (kVp) of 80. Each single 1cm scan slice produced effective radiation dose readings of 0.0024 mSv. The scan area was approximately 13 cm, providing an estimated per scan effective dose of 0.031 mSv of radiation. These dose results were viewed as very positive as one goal of this pilot was to determine how many scans could be conducted in the *in-vivo* study to assess changes in dispersion patterns over time while limiting radiation to a level that has been comparable to other research projects. Based upon this data, subjects could receive up to 20 scans without being exposed to more than 1 mSv of radiation, which is less than one-tenth of a typical single abdominal CT scan.

Discriminatory Capacity

Prior to injection with the iohexol, a scan of the cadaveric thigh was conducted to assess the discriminatory capacity of the proposed scan settings. Tissue discrimination was distinct for skin, subcutaneous tissue, quadriceps muscle groups, femur, and the bone marrow cavity (Figure 12). Upon administration, iohexol could also be viewed in multiple image planes (Figure 13).

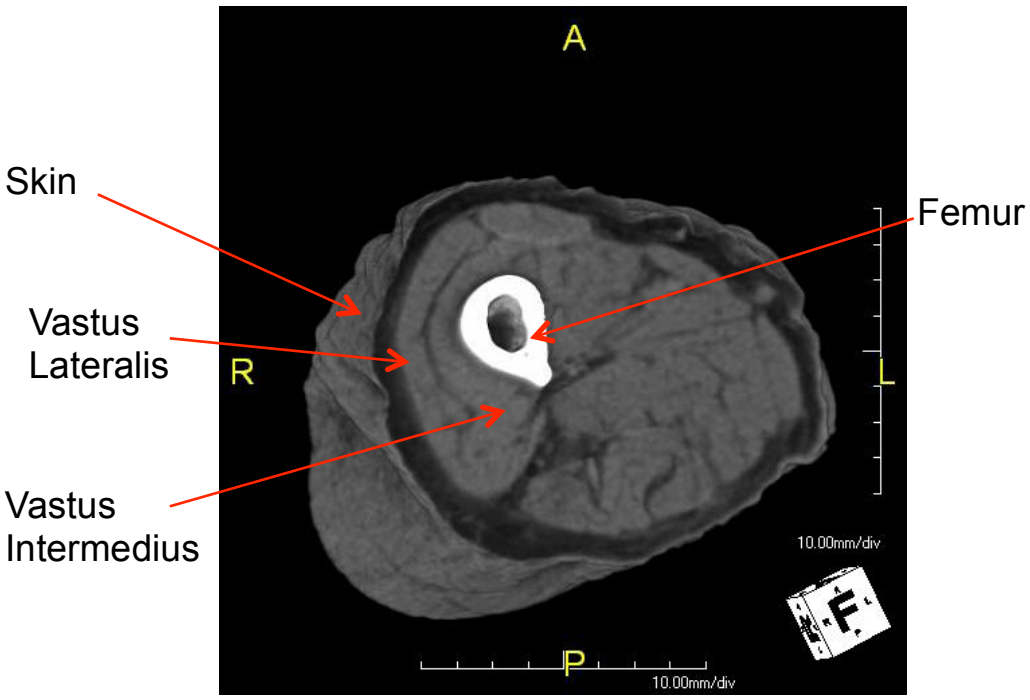
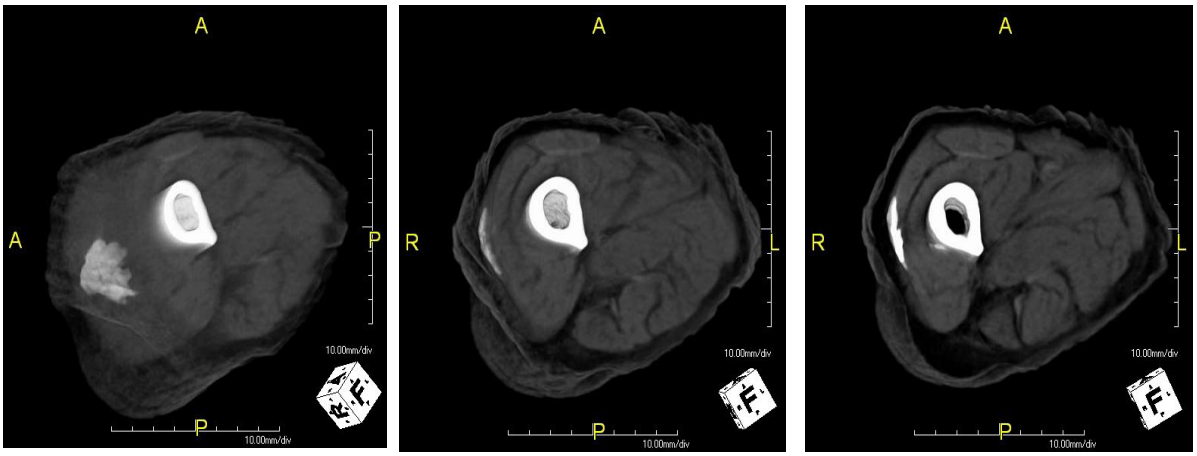


Figure 12: Baseline Scan of Cadaveric Thigh (Near Axial Plane)



Left to right: Oblique, near axial, and axial image planes

Figure 13: Iohexol Administration into Cadaveric Thigh – Multiple Image Planes

Discrimination between intramuscular and subcutaneous injection was obtainable (Figures 14 and 15).

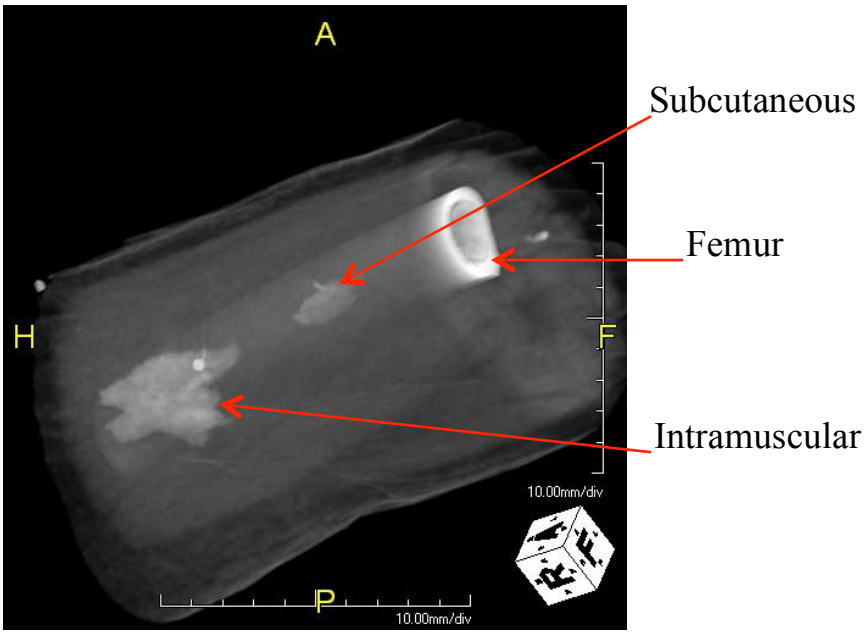


Figure 14: Intramuscular versus Subcutaneous Dispersion of Iohexol in a Cadaveric Thigh (Oblique Plane)

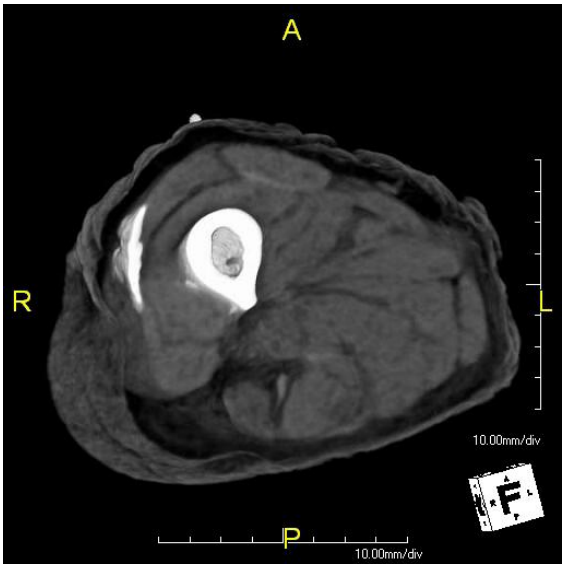
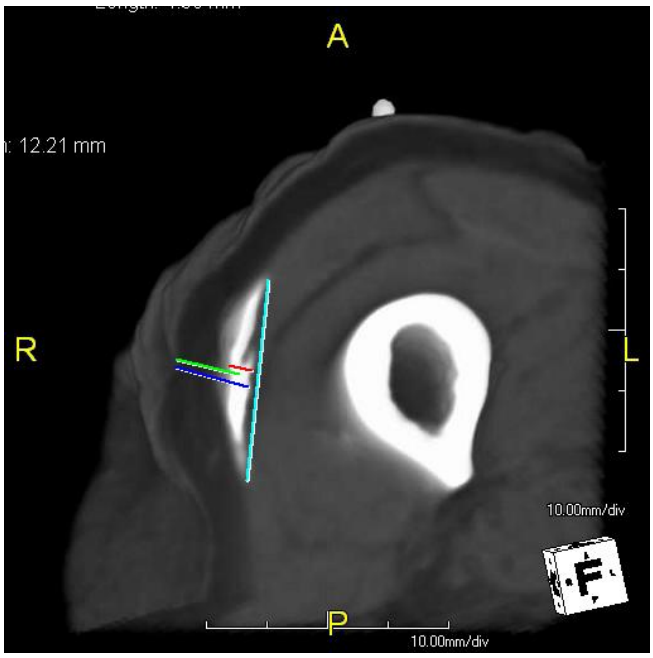


Figure 15: Intramuscular Administration of Iohexol in the Cadaveric Thigh (Near Axial Plane)

Parameters including location, pattern, total depth of dispersion, height of dispersion, width of RCM dispersion, and depth of dispersion to maximum width were distinct and measureable (Figure 16).



Light blue = maximum width of dispersion (33.15mm) , Dark blue = maximum depth of dispersion (13.59 mm), Red = height of dispersion (4.30 mm), Green = Depth of dispersion to maximum width (12.21 mm)

Figure 16: Quantitative Measurement of Iohexol Dispersion

In addition, imaging software was able to reconstruct in three-dimensions, the iohexol administered and provide a measurement of volume administered (Figure 17 and 18). These images can subsequently be animated to provide a fluid perspective on injection location.

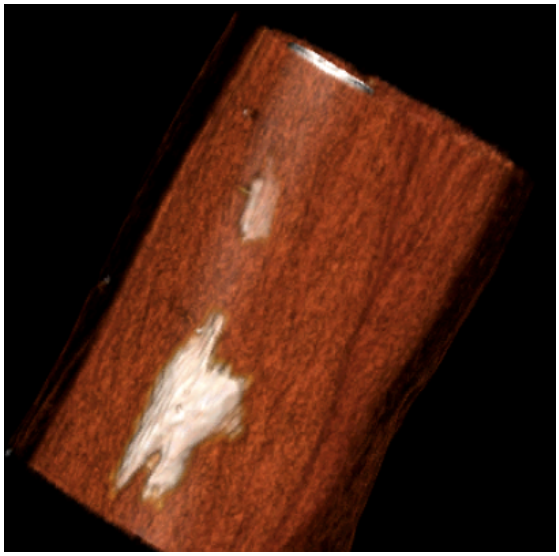


Figure 17: Three-Dimensional Reconstruction of Iohexol Injection in Cadaveric Thigh

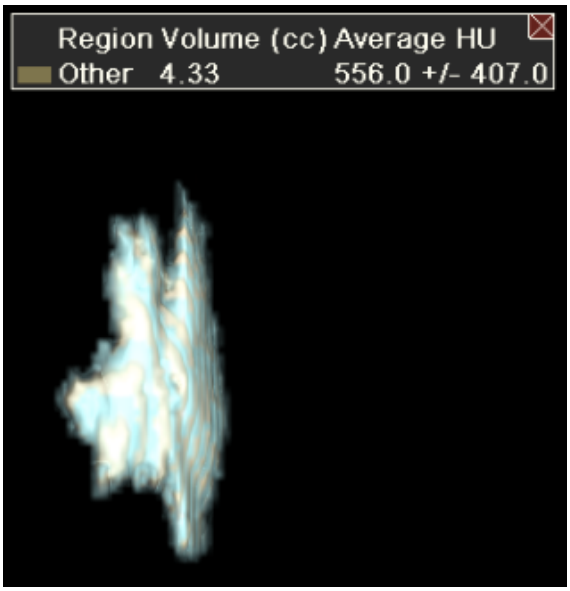


Figure 18: Iohexol Injectate Reconstructed Utilizing Imaging Software allowing for Volume Measurement

Discussion and Conclusion

This pilot study sought to assess Computed Tomography scanning and a tri-iodinated contrast media pharmaceutical as a useable and safe approach for assessing dispersion dynamics that may be introduced by injectable delivery technologies, including pen, jet (needle-free) or auto injectors, and pre-filled syringes. The calculated effective radiation dose was well within desired safety limits per the VCU radiation safety professional team. This allows for follow-up *in-vivo* study planning to include multiple scanning time points that will be able to characterize dispersion immediately following injection and over time. As such, the total radiation exposure for the next human study will be no more than approximately 0.5 to 1 millisieverts (mSv), which is less than the average person receives from background radiation in three to five years. The average person in the U.S. receives an effective dose of about 3 mSv per year from naturally occurring radioactive materials and cosmic radiation from outer space.

The use of the 64-slice CT scanner afforded remarkable tissue detail both pre and post-iohexol administration. The ability to discriminate the borders of the injectate clearly, assess location of dispersion and, most importantly, measure parameters to characterize the dispersion is promising given the previous limited success with other imaging technologies when trying to assess dispersion characteristics. Furthermore, because these measurements can be made and the effective radiation dose is minute, allowing for the ability to scan a subject's thigh over time following dispersion, an assessment of rate of loss or elimination of iohexol from the extravascular compartment to the systemic circulation may theoretically be accomplished. This rate may be compared to typical systemic, intravascular exposure parameters using an assay for iohexol. As such, it is possible to evaluate not only where this particular drug goes following

administration by different delivery technologies, but also to what extent it is deposited and dispersed within the extravascular tissue followed by the extent of absorption and elimination from the vascular compartment. In summary, computed tomography, utilizing iohexol as an injectable standard, is a potentially safe and viable method for assessing dispersion dynamics associated with injectable delivery technologies and should be investigated further in humans.

CHAPTER 4. A RANDOMIZED, SINGLE-BLIND, TWO-TREATMENT, TWO-PERIOD, TWO-SEQUENCE STUDY TO ASSESS COMPUTED TOMOGRAPHY AND THE BIOAVAILABILITY OF IOHEXOL ADMINISTERED SUBCUTANEOUSLY BY TWO DELIVERY SYSTEMS IN HEALTHY, HUMAN VOLUNTEERS

Introduction and Overview

This study consisted of two components. The first component included a comparison of *in-vivo* injection dispersion characteristics from an auto-injector versus pre-filled syringe delivery system, administered subcutaneously (depending on subject body habitus), using a non-ionic radiocontrast media, iohexol (Omnipaque®) and computed tomography (CT) scanning. The second component involved a comparison of the bioavailability of iohexol delivered by an auto-injector delivery system and iohexol delivered by a pre-filled syringe delivery system. Both of these components were assessed by conducting a randomized, single-dose, cross-over study.

The Study occurred at the Clinical Research Center (CRSU) and Radiology Department at the Virginia Commonwealth University Health System. Administration of both treatment regimens (Auto-injector with Iohexol and Pre-filled Syringe with Iohexol) occurred in the anterolateral thigh of each subject using the same needle length and needle gauge. The systemic exposure metrics of iohexol were determined by plasma and urine sampling. Injectable dispersion characteristics were assessed using iohexol and CT scanning administered in the

thigh. Safety was assessed by adverse events (AEs), clinical laboratory tests, vital signs, and physical examinations.

Twelve eligible subjects were enrolled in the study, and randomized to one of two treatment sequences as shown in Table 3 below.

Table 3: Overview of Study Design

Treatment Sequence	Number of Subjects	Period 1	Period 2
1	Up to 6	Tx A (RCM PFS SC)	Tx B (RCM Auto SC)
2	Up to 6	Tx B (RCM Auto SC)	Tx A (RCM PFS SC)

Subjects were dosed on Day 1 in each study period.
RCM = Radiocontrast Media (Iohexol), Auto = Auto-injector
PFS = Pre-filled Syringe, SC = Subcutaneous

In each of the treatment periods, subjects received either a subcutaneous injection of iohexol administered using an auto-injector in the mid-anterolateral region of the thigh (Autoject® 2, Owen Mumford, Ltd.), or an injection of iohexol administered using a pre-filled syringe (Terumo Medical Corporation) and needle in the mid-anterolateral region of the thigh. Prior to administration, subjects received a CT scan of the thigh region to be injected as well as the collection of baseline plasma and urine samples. Following administration, additional CT scans occurred to provide an assessment of delivery location and plasma samples were collected to assess the bioavailability of iohexol. There was a washout period of at least 7 days between treatment periods.

Ethics

Institutional and Radiation Safety Review Boards

The clinical study protocol, any amendments, subject information sheets, written informed consent forms (ICFs), and all other relevant study documentation were reviewed and

approved by the responsible Institutional Review Board (IRB; Virginia Commonwealth University, 800 East Leigh Street, PO Box 980568, Richmond, VA 23298) as well as the VCU Center for Clinical and Translation Research Protocol Review Committee (Clinical Research Services Unit, North Hospital, 8th Floor, 1300 East Marshall Street, PO Box 980155, Richmond, VA 23298). The IRB assigned protocol identification code HM13424 to this investigation. Additionally, radiation dosimetry results from the cadaveric study described in Chapter 3 as well as the protocol for computed tomography scanning were reviewed and approved by a Radiation Safety Review Officer (Office of Environmental Health and Safety- Radiation Safety Section, 1101 E. Marshall St. PO Box 980112, Richmond, VA 23298). A copy of the IRB and Radiation approval forms are provided in Appendix A.

Ethical Conduct of the Study

This study was designed and monitored in accordance with procedures which comply with the ethical principles of Good Clinical Practices and in accordance with the Declaration of Helsinki.

Subject Information and Consent

All subjects were informed of the nature and purpose of the study, and their written informed consent was obtained prior to the pre-study screening procedures conducted within 30 days prior to the first dosing day. A sample of the written ICF is provided in Appendix B.1. Informed consent was also verified independently by at least two study investigators and/or study nurses prior to entry into the study utilizing the process documentation form provided in Appendix B.2.

Investigators and Study Administrative Structure

Principal Investigator (PI)

William R. Garnett, Pharm.D.

VCU School of Pharmacy

Sub-Investigator

William H. Barr, Pharm.D., Ph.D.

VCU School of Pharmacy

Assistant Research Coordinator

Annmarie Panchem

VCU School of Pharmacy

Project Manager and Student Investigator

Eric S. Edwards, BS

VCU School of Pharmacy

Clinical Research Unit

Lead Study Nurse: Lou Usry, R.N.

Virginia Commonwealth University (VCU) Clinical Research Services Unit (CRSU) at the VCU Health System (VCUHS)

Richmond, VA

Medical Monitor:

John N. Clore, M.D., M.S.

Virginia Commonwealth University CRSU at the VCUHS

Richmond, VA

Computed Tomography Services

Radiology Coordinator: Megan Quinn, Radiology Oversight: Jonathan Ha, M.D.

VCUHS Department of Radiology

Richmond, VA

Central Laboratory Facilities

Clinical Laboratory Tests:

Coordinator: Millicent Smith

VCUHS Clinical Pathology Research Services (CPRS)

Richmond, VA

Bioanalytical Laboratory:

Analytical Services Coordinator: Matthew Halquist

VCU Bioanalytical Core Laboratory Service Center

Richmond, VA

Study Materials and Management

VCU Medical Center Investigational Drug Service

Department of Pharmacy Services

Richmond, VA

All persons involved at the clinical site were qualified to perform their roles. The curricula vitae of the Principal Investigators and Medically Responsible Investigator are provided in Appendix C. This research project was supported in-part by award Number UL1RR031990 from the National Center for Research Resources. The content described in this

investigation is solely the responsibility of the author and does not necessarily represent the official views of the National Center for Research Resources of the National Institutes of Health.

Study Aims

Aim 1

To evaluate Computed Tomography (CT) as an approach for investigating sources of variability in injectable delivery systems and to assess the dispersion of an injectate beneath the dermis over time with its relative bioavailability using iohexol, a type of radiocontrast media (RCM) as the injectable standard.

Aim 2

To assess the discriminatory capacity of CT for predicting bioavailability by investigating the inter-individual and intra-individual variability in injectable dispersion using two distinct injectable delivery systems.

Investigational Plan

Overall Study Design and Plan Description

This study was a randomized, single dose, single-blind, 2-treatment, 2-period, 2-sequence crossover study to document the tissue bioavailability and systemic exposure of iohexol delivered by a pre-filled syringe and a commercially available auto-injector delivery system. Twelve eligible subjects were planned for enrollment and randomization to one of two treatment sequences pre-dose on Day 1 of Period 1, according to a randomization schedule prepared by the VCU Health System Investigational Drug Service before the start of the study. Subjects were randomized to a treatment sequence; AP or PA, as previously described in Table 3.

Interim analysis. In order to assess whether the approach provided useable data, an interim analysis was planned following at least two subjects completing the full study protocol. This interim analysis resulted in minor changes in plasma sampling times as well as CT scanning time points as described in the **Interim Analysis** results section.

Treatment sequences. During Screening (Day -30 to Day -1), subjects signed informed consent forms and underwent procedures to determine eligibility. Eligible subjects reported to the CRSU on Day 1 (for all treatment periods), the day of dose administration, and underwent pre-dose procedures. On Day 1 of each treatment period, subjects received a single injection of investigational product in the thigh, administered by the same, trained study nurse. Subjects were discharged from the CRSU after the post-dose blood sample was collected (Periods 1 and 2). Subjects remained at the CRSU overnight after dosing during any period at the discretion of the Investigator. There was a wash-out period of at least 7 days between treatment periods.

The systemic exposure of iohexol delivered by either the pre-filled syringe or auto-injector was determined by plasma concentrations collected through the post-dose sampling time. Intensive sampling was obtained during the first hour post-dose to fully characterize the early exposure profile after product administration. All CT scans occurred within this first hour in order to evaluate early dispersion characteristics. Due to study logistic limitations, CT scanning beyond the first hour was unobtainable. Safety was assessed by clinical laboratory tests, physical examinations, monitoring of vital signs and monitoring of adverse events (AEs). Refer to Table 4 for a study flowchart with the schedule of assessments.

Table 4: Study Schedule of Assessments

Evaluation	Screening Day -30 to Day -1	Treatment Period 1		Treatment Period 2	
		Day 1 pre-dose[a]	Day 1 treatment	Day 1 pre-dose	Day 1 treatment
Informed consent	X				
Eligibility Criteria	X	X		X	
Medical history	X				
Urine pregnancy test [b]	X	X		X	
Physical examination	X				
Clinical laboratory tests (Serum Chemistry and Hematology)	X				
Vital signs	X	X	X (at discharge)	X	X (at discharge)
Study treatment including iohexol administration and CT Scanning			X		X
Blood samples for systemic exposure analysis			X		X
Urine samples for systemic exposure analysis [d]			X		X
Monitor/record AEs and concomitant medications [e]		X	X	X	X

[a] Admission to clinical site prior to dosing, [b] All women, regardless of childbearing potential, [c] Vital signs and ECGs were collected within 60 minutes pre-dose [d] Subjects were asked to provide urine samples in a specimen container, [e] Included the review of medications taken since Screening as well as any change of health status since Screening.

Discussion of Study Design

A 2-period, 2-sequence crossover design with the Auto-injector administering iohexol once and pre-filled syringe administering iohexol once in a random sequence was selected to allow for comparisons of the systemic exposure and dispersion profiles of iohexol administered using both delivery systems between subjects and within subjects. All subjects were required to fast for a minimum of 4 hours before dosing (to mitigate, for example, food-related effects that may influence the absorption behavior of iohexol). A washout period of at least 7 days was considered sufficient to prevent carryover effects of the two delivery systems based upon what was known regarding the pharmacokinetics of iohexol at the time of the study. Subjects were blinded to study treatment to minimize bias based on subjective expectations. For example, a subject being able to view the delivery system prior to administration may have resulted in variations in anxiety that could affect the absorption of iohexol (e.g. an endogenous epinephrine response resulting in vasoconstriction at the injection site due to anxiety). Routine safety assessments (AE monitoring, vital signs, 12-lead ECGs, 2-lead cardiac telemetry, physical examinations, clinical laboratory tests and concomitant medication monitoring) were performed per usual measurements in a bioavailability study of this nature by study personnel. Serial blood sampling from pre-dose up to 10 hours post-dose and urine sampling from pre-dose to 24 hours post-dose was considered sufficient to determine iohexol systemic exposure profiles following an injection using either delivery system.

Selection and Recruitment of Study Population

Twelve healthy, adult, male or female volunteers who met all of the entry requirements were planned for enrollment in the study. Subjects were recruited using approved fliers on the academic and medical campuses of Virginia Commonwealth University. At the completion of

each treatment period, subjects were offered $150.00 for their participation. If a subject completed both treatment periods, they received an additional $100.00 for a total compensation of $400.00 for full participation in the study.

Inclusion Criteria

For inclusion into the trial, subjects were required to fulfill all of the following criteria:

1. Healthy adult male and female subjects between 21 and 55 years (inclusive)
2. Ability to give written informed consent to participate in the study
3. Body mass index (BMI) between 18 and 30 kg/m², inclusive, and a weight of ≥ 50 kg

Exclusion Criteria

Any of the following was regarded as a criterion for exclusion from the trial:

1. Unable to read
2. Known allergies to radiocontrast media
3. Not fluent in English
4. Female subjects who were trying to conceive, were pregnant, or were lactating
5. Positive urine pregnancy test prior to each drug administration for all women, regardless of childbearing potential
6. A history of clinically significant pulmonary, immunologic, psychiatric, or cardiovascular disease or any other condition which, in the opinion of the Medical Investigator, would jeopardize the safety of the subject or impact the validity of the study results
7. Currently (in the last 7 days) taking any prescription or non-prescription medicines (excluding oral contraceptives), vitamins, dietary or herbal supplements
8. Previous history of abuse or recent use of alcohol or illicit drugs

9. Subjects who donated blood within 30 days or plasma within 14 days of the first study dosing
10. Participation in a clinical trial within 30 days prior to study initiation

Exclusions were meant to ensure the population studied was able to understand the procedures and risks associated with the study, to ensure that all subjects were healthy volunteers, and were not placed at unnecessary risks based upon the research proposal. This included ensuring subjects had not donated blood since blood samples will be drawn, were not pregnant to ensure no potential harm to a fetus, and had no history of being allergic to the investigational products.

Removal of Subjects from Therapy or Assessment

Subjects were informed that they had the right to withdraw from the study at any time for any reason without prejudice to their medical care. Subjects were withdrawn from the study for any of the following reasons:

1. Subject request
2. Subject was unwilling or unable to comply with the protocol
3. Medical reason, at the discretion of the investigator and/or the Medical Monitor

The reasons for discontinuation of the investigational product and/or subject withdrawal were recorded if any subject were to withdraw from the study. The PI was to notify the Medical Monitor immediately when a subject was discontinued/withdrawn due to an AE. All subjects who were withdrawn from the study should have completed the tests and evaluations scheduled for the last study day at the time of withdrawal. In the case of subject withdrawal from the study, due to the nature of this investigation, additional subjects were not to be enrolled to complete the study.

Pharmaceutical Product Administration

Treatments Administered

During each treatment period subjects received either a single, subcutaneous injection of iohexol (150 mgI/mL) or iohexol (300 mgI/mL) administered using the pre-filled syringe or auto-injector delivery system as described below.

Identity of Investigational Pharmaceutical Product

As discussed in Chapters 2 and 3, radiographic contrast media are utilized clinically in CT scanning to improve the visibility of internal body structures in various imaging techniques. Because of its success in providing direct visualization of dispersion in the cadaveric study and due to the availability of a modifiable assay that has previously been used at VCU for another investigation, iohexol (Omnipaque®, General Electric Company- Healthcare Division) was chosen as the pharmaceutical product for this investigation. Iohexol (Omnipaque) is a tri-iodinated, low osmolar, safe and effective, nonionic, water-soluble contrast medium that is well established, with U.S. clinical experience since 1985. Worldwide, more than 100 million doses of iohexol have been administered. This radiocontrast media agent is approved for use in adults and children and is indicated for a broad range of intravascular diagnostic procedures such as coronary angiography, spinal cord imaging, and body cavity procedures including shoulder and knee joints.

Clinical pharmacology and pharmacokinetics of Omnipaque (Iohexol). Following intravascular injection, iohexol is distributed in the extracellular fluid compartment and is excreted unchanged by glomerular filtration. It will opacify those vessels in the path of flow of the contrast medium permitting radiographic visualization of the internal structures until significant hemodilution occurs. Approximately 90% or more of the injected dose is excreted

within the first 24 hours, with the peak urine concentrations occurring in the first hour after administration. Plasma and urine iohexol levels from 500 mgI/kg to 1500 mgI/kg does not significantly alter the clearance of the drug. The following pharmacokinetic values were observed following intravenous administration of iohexol (between 500 mgI/kg to 1500 mgI/kg) to 16 adult human subjects: renal clearance—120 (86-162) mL/min; total body clearance—131 (98-165) mL/min; and volume of distribution—165 (108-219) mL/kg (Omnipaque, 2008).

Renal accumulation is sufficiently rapid that the period of maximal opacification of the renal passages may begin as early as 1 minute after intravenous injection. Urograms become apparent in about 1 to 3 minutes with optimal contrast occurring between 5 to 15 minutes. In nephropathic conditions, particularly when excretory capacity has been altered, the rate of excretion may vary unpredictably, and opacification may be delayed after injection. Severe renal impairment may result in a lack of diagnostic opacification of the collecting system and, depending on the degree of renal impairment, prolonged plasma iohexol levels may be anticipated. In these patients, as well as in infants with immature kidneys, the route of excretion through the gallbladder and into the small intestine may increase. Iohexol displays a low affinity for serum or plasma proteins and is poorly bound to serum albumin. No significant metabolism, deiodination or biotransformation occurs. Animal studies indicate that iohexol does not cross an intact blood-brain barrier to any significant extent following intravascular administration (Omnipaque (Iohexol) Prescribing Information. General Electric Company. 2008).

Omnipaque enhances computed tomographic imaging through augmentation of radiographic efficiency. The degree of density enhancement is directly related to the iodine content in an administered dose; peak iodine blood levels occur immediately following rapid intravenous injection. Blood levels fall rapidly within 5 to 10 minutes and the vascular

compartment half-life is approximately 20 minutes (Olsson, Aulie, Sceen, & Andrew, 1983). This can be accounted for by the dilution in the vascular and extravascular fluid compartments which causes an initial sharp fall in plasma concentration. Equilibration with the extracellular compartments is reached in about ten minutes; thereafter, the decline becomes exponential. The pharmacokinetics of iohexol in both normal and diseased tissue (such as tumors) has been shown to be variable.

Contrast enhancement appears to be greatest immediately after bolus administration (15 seconds to 120 seconds). Thus, greatest enhancement may be detected by a series of consecutive two-to-three second scans performed within 30 to 90 seconds after injection i.e. dynamic computed tomographic imaging. Utilization of a continuous scanning technique i.e. dynamic CT scanning, may improve enhancement and diagnostic assessment of tumor and other lesions such as abscess, occasionally revealing unsuspected or more extensive disease. For example, a cyst may be distinguished from a vascularized solid lesion when pre-contrast and enhanced scans are compared; the non-perfused mass shows unchanged x-ray absorption (CT number). The physicochemical properties of the drug may be found in Table 5 below.

Table 5: Physicochemical Properties of Iohexol 300

mg iodine/ml	300
Viscosity (cps)	
at 20°C	11.8
at 37°C	6.3
Osmometrics at 37°C	
Osmolality (mOsm/kg)	672
Specific Gravity (g/mL)	1.345
pH	7 ± 0.5
Molecular Formula	$C_{19}H_{26}I_3N_3O_9$
Molecular Weight	821.14 (iodine content 46.36%)
Melting Point	174-180°

Iohexol is designated chemically as N,N′ - Bis(2,3-dihydroxypropyl)-5-[N-(2,3-dihydroxypropyl)-acetamido]-2,4,6-triiodoisophthalamide.

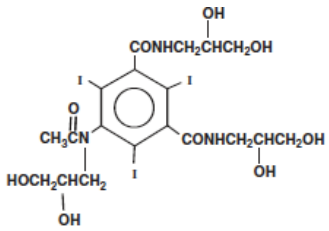


Figure 19: Structural Formula of Iohexol

Each milliliter of iohexol solution contains 1.21 mg of tromethamine and 0.1 mg of edetate calcium disodium with the pH adjusted between 6.8 and 7.7 with hydrochloric acid. All solutions are sterilized by autoclaving and contain no preservatives (Omnipaque, 2006).

Identity of Investigational Delivery Systems

There are a wide-array of commercially available auto-injectors; however, most come pre-filled with a pharmaceutical product preventing easy access to the drug container closure system for modification or filling with a custom injectable, such as a contrast media agent. In order to limit the source of variability being assessed at the point of injection to device-related factors only (e.g. force/mechanics of injection), an FDA-approved auto-injector was chosen that allowed for the insertion of a standard pre-filled syringe into the delivery system. This allowed for the comparison of a manual delivery technique using a pre-filled syringe device to an automatic injection using an auto-injector without the introduction of additional variables that could lead to challenges in assessing usability.

The VCU Health Center Investigational Drug Service obtained and dispensed all study materials, including the syringes pre-filled with iohexol by the Drug Service staff per standard

operating procedures, with the exception of the auto-injector, which was provided by the study investigators. All supplies of investigational product were stored at room temperature. Until dispensed to nursing personnel for dosing procedures, investigational product was stored in a secure area, accessible to authorized persons only. Accountability for investigational product was the responsibility of the PI.

Auto-injector selection. All subjects were dosed with the Autoject® 2 (Owen Mumford, Ltd., Lot: JMD2009-1). This auto-injector system is a re-usable automatic injection device designed to incorporate a wide-array of plastic and glass fixed-needle syringes. The product is indicated for the subcutaneous administration of insulin and a variety of other injectables (Figure 19). It also includes an adjustable depth setting mechanism. This depth setting mechanism was adjusted to ensure the exposed needle length mimicked the exposed needle length of the pre-filled syringe to be administered manually.

Figure 20: Autoject® 2 Delivery System

Pre-filled syringe delivery system selection. A 1mL 23 gauge x ½" fixed-needle syringe (Terumo Sursaver™, Terumo Medical Corporation, Lot MM1936-09) was chosen for this investigation based upon its ability to be used with the auto-injector delivery system (Figure

20). The syringe was pre-filled with either 0.5mL or 1.0ml iohexol (300mgI/mL) by the VCUHS Investigational Drug Service.

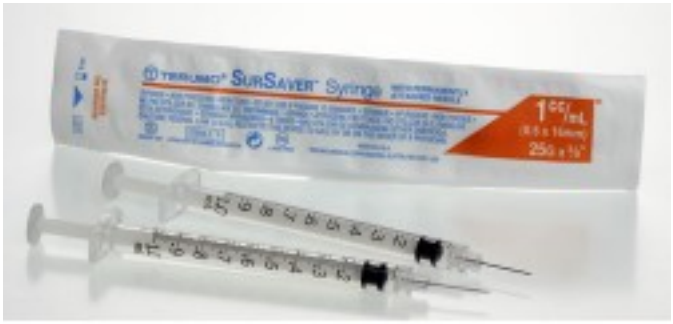


Figure 21: Terumo Sursaver Syringe Example

Method of Assigning Subjects to Treatment Groups

Enrolled subjects were allocated to one of 2 treatment sequences according to a computer generated randomization schedule prepared by the VCU Health Center Investigations Drug Service prior to the start of the study. The randomization schedule included 3-digit subject numbers. Once a randomization number was allocated to one subject, it could not be re-assigned to another subject. The randomization schedule is located in Appendix D.

Selection of Dose and Regimen in the Study

In this crossover-design study, in each treatment period, subjects received either a single SC injection of 300 mg (1.0 mL) iohexol or injection of 150 mg (0.5 mL) iohexol using the auto-injector or a single SC injection of 300 mg (1.0 mL) iohexol or 150 mg (0.5 mL) iohexol administered using the pre-filled syringe in the mid-anterolateral thigh region (i.e., the measured midpoint between the upper border of the patella [knee cap] and the inguinal fold [crease] at top of thigh). All injections occurred no less than 1 and no more than 2 inches from the previous injection site. This was accomplished by marking the injection site and measuring with a ruler

from the marked location. The larger dose of 300 mg (1.0 mL) was only utilized in this study if the planned interim analysis demonstrated the 150 mg dose to be insufficient in direct visualization by computed tomography or the plasma concentration analysis fell below the limit of quantitation for the chosen analytical method. Water was allowed and encouraged *ad libitum* during the study. Subjects were required to fast for 4 hours after dosing. Standard meals were provided at approximately 4 hours after administration of the investigational product. During housing, meal plans were identical for all treatment periods.

Blinding

This was a single-blind study; subjects were blind to their own injections and to the injections received by other subjects. Subjects were also blinded from seeing which delivery system (auto-injector or pre-filled syringe) was being administered during their own injections. To ensure proper blinding, a screen was placed on top of the subject's mid section to prevent viewing of the injection. It was not possible to blind the nurse administering the drug as injection systems were visibly different.

Prior and Concomitant Therapy

Concomitant medications were reviewed on Day 1 of each Treatment Period. This included the review of medications taken since screening to ensure inclusion/exclusion criteria had been met for the study.

Treatment Compliance

Trained CRSU personnel administered the investigational product. The same individual at the CRSU administered all injections to all subjects. The date and time of the injection as well as the location were recorded for each subject at each treatment period on the Subject Flow Sheets (See Appendix E.1a – interim flowsheet and Appendix E.1b – final flowsheet). If an

injection system malfunctioned or was accidentally activated prior to injection of the subject, a description of the incident was to be recorded, and a new device was to be obtained from the pharmacy. Finally, subjects were under the direct supervision of the CRSU staff during the treatment periods to ensure compliance with the treatment regimen.

Computed Tomography, Systemic Exposure and Safety Variables Assessed

Appropriateness of Measurements

The safety measures used during this study are standard accepted methods of monitoring the safety of subjects during an investigative clinical trial and took into account the research-based use of computed tomography scanning, pharmacologic properties of iohexol being studied, as well as the locations of study activities. All safety assessments were carried out according to the standard operating procedures (SOPs) of the CRSU. The systemic exposure variables that were utilized were appropriate to characterize the plasma concentration-time profiles for iohexol injection administered by the auto-injector and pre-filled syringe. Parameters obtained from Computed Tomography scanning included the qualitative variable of location of injection as well as novel quantitative measures that were consistent with those that were appreciated through the cadaveric pilot study work conducted as described in Chapter 3.

Iohexol Concentration Measurements

Iohexol assay. An HPLC-UV method was utilized for the determination of iohexol in human plasma and urine as described by Farthing *et al*. (Farthing, D., Sica, Larus, Ghosh, Farthing, C., Vranian, & Gehr, 2005). This method incorporating iohexol was originally developed for assessment of Glomerular Filtration Rate (GFR) from medications marketed for patients with cardiovascular disease. Although the method was used for investigations involving

the intravenous administration of iohexol, the results indicated that it was possible to achieve detection at levels as low as 2.5 µg/mL, which the investigators felt could be sufficient for the analysis of iohexol administered subcutaneously.

Chemicals used. Iohexol (Omnipaque) was purchased from United States Pharmacopeia (USP, Rockville, MD, USA) (South Plainfield, NJ, USA). Trifluoroacetic acid was reagent grade, methanol and acetonitrile were HPLC grade and all were purchased from VWR (Radnor, PA, USA).

Equipment and mobile phase. The HPLC equipment consisted of a Waters 2695 Separations Module Alliance (Waters Corporation, Milford, MA, USA). The analytical column used was a Supelco Discovery C_{18}, 250mm x 4mm i.d., 5µm packing, 180 Å (Supelco, Bellefonte, PA, USA). The mobile phase consisted of aqueous trifluoroacetic acid (0.1% TFA in deionized water (pH 2.2), v/v) and methanol gradient. An injection volume of 10 µL of the prepared plasma sample and 20 µL of the prepared urine sample was accomplished using the Waters 2695 Separations Module Alliance. Component detection was achieved using the Waters 2487 dual wavelength absorbance detector Waters Corporation, Milford, MA, USA with an absorbance wavelength of 254nm. Data acquisition and component computations were performed using Empower Pro software Waters Corporation, Milford, MA, USA.

Sample preparation. Plasma samples were thawed and prepared by pipetting 250 µL of plasma and 250 µL of 0.1% TFA in deionized water into a polypropylene bullet centrifuge tube. Plasma proteins were precipitated by vortexing for 15 s. The samples were centrifuged at 13,000 rpm for 10 min. The clear supernatant was transferred to a 0.2 µm Nanosep MF filter (Pall Corporation, Port Washington, NY, USA) and centrifuged for 1 minute at 13,000 rpm. The filtered supernatants were transferred to glass HPLC autosampler vials. Urine samples were

prepared by pipetting 20 µL of urine and 980 µL of deionized water directly into the glass autosampler vial and vortexing for 10 s. For urine and plasma sample analysis, 10 and 20 µL were injected into the HPLC system, respectively.

Linearity, limit of quantitation and detection, accuracy, precision and recovery. The plasma method was linear throughout the concentration range 2.5 -100 µg/mL (mean correlation coefficient of 0.9947, n=9). The iohexol isomers are separated under a lower column temperature (~20) however, by increasing the column temperature to 40 °C the isomers will co-elute for improved sensitivity (Farthing *et al*., 2005). Both isomers (labeled I2 and iohexol) were monitored during quantification. Accuracy and precision for the method was determined by evaluation of replicate prepared control samples. The method demonstrated good accuracy and precision for both plasma and urine samples. Precision and accuracy was assessed for both matrices and found to be acceptable (±15%) according to FDA bioanalytical guidelines (FDA, 2001). The quality control precision and accuracy results as well as the back-calculated values for the calibration curve standards may be found in Appendix F.

Chromatography. The method demonstrated good plasma chromatographic selectivity with no endogenous interference at the retention time of approximately 6.14 min. A sample chromatogram may be found in Appendix G. Urine chromatograms displayed good detector response and adequate baseline resolution from the endogenous urine substances.

Plasma sampling procedure. Plasma samples for the first two subjects were drawn at pre-dose, 10, 20, 30, 40, 50 minutes and 1, 2, 3, 5, and 7 hrs. Following the interim analysis as previously described, the sample schedule was modified and approved by the IRB to drawing times at pre-dose, 15, 30, 40 minutes and 1, 1.5, 2, 4, 6, 8 and 10 hrs post-dose. Subjects were

asked to provide urine samples in a specimen container. Urine samples were collected at pre-dose, 0-2, 2-3, 3-4, 4-6, 6-8, and 8-24 hours post-dose intervals.

An in-dwelling catheter for multiple blood draws was inserted into the subject and cared for according to the CRSU SOPs. In the event that the catheter did not function properly, a needle was used to collect blood samples and was recorded if required. All blood samples (1 × 10 mL) were collected in Heparin/Lithium Vacutainers.

The blood sample was immediately transported to the laboratory for processing following sample collection. Sample processing initiated within 60 minutes of blood collection and consisted of separating the plasma by centrifugation at ~3000 rotations per minute (rpm) × 10 minutes at 4°C and transferring equal aliquots of plasma to 2 clearly labeled polypropylene tubes. One tube was considered the primary sample, and the other tube was considered the back-up sample. Both the primary and back-up plasma samples were immediately stored in a non-defrosting -20°C freezer. The plasma samples were analyzed for concentrations of iohexol using the high performance liquid chromatography method (HPLC) as described above.

The following systemic exposure parameters were estimated from the plasma and urine iohexol concentrations:

- C_{max}: maximum plasma or urine concentration
- T_{max}: time to maximum plasma or urine concentration
- $AUC_{(0-t)}$: area under the concentration-time curve from baseline to the last measurable concentration
- $AUC_{(0-\infty)}$: area under the plasma concentration-time curve from baseline extrapolated to infinity

- $AUC_{(partial)}$: area under the plasma concentration-time curve from baseline extrapolated to select sample time points prior to the last measurable concentration
- $AUC_{(Tmax)}$: area under the plasma concentration-time curve from baseline extrapolated to select to the time of maximum plasma concentration
- λz: elimination rate constant
- $T_{½}$: terminal elimination half-life

Computed Tomography (CT) Parameter Measurements

All subjects received CT scanning in the VCU Health System Radiology Department using the Somatom Sensation®, 64-slice CT scanner. An initial scan of the thigh region from approximately 3 inches below the pubis symphysis to approximately 2 inches above the patella (approximately 13 cm total scan length of the extremity) was conducted to localize the injection area and to provide baseline measurements prior to dosing with iohexol. CT image collection time points included 0.5, 5, 10, 15 and 20 minutes for the first two subjects and 0.5 10, and 20 minutes for the subsequent 10 subjects following the interim analysis results. More detail for this IRB-approved protocol amendment may be found in the Interim Analysis section below.

Subject CT scans were processed utilizing iSite Picture Archiving and Communications System (iSite PACS, Philips Koninklijke Electronics, ver. 3.6.52) software. Images were enhanced using the Laplacian Method to increase the visibility of edges in each image slice. The following parameters were measured for each subject, at each scanning time point, for each period from the computed tomography images and associated iSite PACS:

- location of Injection
- maximum depth of dispersion (MDd)
- maximum width of dispersion (MWd)

- depth at maximum width of dispersion (DMWd)

In addition, the iSite PACS includes a tool for measuring radiodensity. This tool measures radiodensity in Hounsfield Units (*HU*), which represents a line transformation from linear attenuation coefficient measurements into one where water is assigned a value of zero and air is assigned a value of -1,000. The linear attenuation coefficient is the probability that an X-ray photon will interact with the material it is traversing per unit path length travelled. If *mw*, *ma*, and *m* are the linear attenuation coefficients of water, air and a substance of interest, the *HU* of the substance of interest is:

$$HU = 1000(m - mw)/(mw - ma)$$

Thus, a change of one Hounsfield unit (*HU*) corresponds to 0.1% of the attenuation coefficient difference between water and air, or approximately 0.1% of the attenuation coefficient of water since the attenuation coefficient of air is nearly zero. This allows for radiodensity in this study to provide a measure of iohexol elimination from the injection site in the extravascular tissue compartment. The change in radiodensity over time at the injection site can subsequently provide an estimate of the iohexol loss rate from the extravascular compartment into the systemic circulation.

Safety Variables

Safety was assessed by clinical laboratory tests, physical examinations, urine pregnancy testing, 12-lead ECG at screening, monitoring of vital signs, and monitoring of AEs and concomitant medications.

Adverse events. Subjects were queried regularly on all study days using non-leading questions, such as “How do you feel?” In addition, all AEs reported spontaneously during the course of the study were recorded. Adverse event description details included start date and time,

stop date and time, severity, relationship to investigational product, action taken, outcome and whether it was serious. The severity of AEs was rated as mild, moderate, severe or life threatening. Relationship to investigational product was indicated as none, unlikely, possible, probable, or definite. Outcome was recorded as resolved, ongoing, death or unknown.

Action taken regarding an AE was recorded and indicated as none, investigational product withheld permanently, medication given, or other. Details of any medication given were recorded and included: medication name, start date and time, stop date and time, dose, route, frequency, reason and whether it was ongoing at the end of the study. Details of 'other' were specified. A sample Adverse Event Form is located in Appendix E.2.

Clinical laboratory tests. Blood samples for clinical laboratory testing (hematology, chemistry) were obtained at Screening. Following blood and urine collection, samples were delivered to the VCUHS Clinical Pathology Research Services (CPRS) for analysis.

Hematology. The following hematology parameters were assessed: Hematocrit, hemoglobin, white blood cell (WBC) count, differential white blood cell count, red blood cell (RBC) count, and platelets.

Chemistry. The following clinical chemistry parameters were assessed: Alkaline phosphatase, aspartate transaminase (AST), alanine aminotransferase (ALT), total bilirubin, creatinine, glucose, blood urea nitrogen (BUN), sodium, potassium, chloride, calcium, anion gap, total protein, albumin, and globulin.

Additional clinical laboratory tests. At screening and prior to each treatment (CT Scan), urine was collected from female subjects for urine pregnancy testing using QuickVue One-Step hCG urine testing (Quidel Corporation).

Vital signs. Vital signs were assessed at Screening and at the following time points on each treatment day (Day 1 of each treatment period): within 60 minutes of dosing (pre-dose) and at approximately 6 hours post-dose. The following vital signs were measured:

- supine blood pressure (mmHg);
- heart rate (beats per minute [bmp]);
- oral temperature (°C);
- respiratory rate (breaths per minute).

Vital signs were performed according to the applicable CRSU SOPs. Supine blood pressure recordings were made after the study subject had been recumbent and at rest for ≥ 5 minutes. A sample Screening Form displaying vital sign measurements may be found in Appendix E.3.

12-Lead Electrocardiograms. Standard 12-lead ECGs were performed at Screening by CRSU study nurses. Electrocardiograms were performed after the subject had been resting supine for ≥ 5 minutes. Electrocardiograms were also evaluated by a qualified physician for the presence of abnormalities (qualitative assessment). The physician assessed each ECG as normal, abnormal/not clinically significant (NCS), or abnormal/clinically significant (CS).

Physical examinations. Each subject received a complete physical exam at Screening. The physical examination included an assessment of general appearance and a review of systems (skin, eyes/ears/nose throat, head/neck/thyroid, lymphatic, lungs/chest, cardiovascular, abdomen, genitourinary, extremities, neurological and musculoskeletal). Additionally, the Screening exam included the following measurements: weight (kg), height (cm), thigh circumference (cm) and a calculation of body mass index (kg/m^2). Thigh circumference and skin-fold thickness were measured at the mid-point of the anterior (front) surface of the thigh, midway between the patella

(knee cap) and inguinal fold (crease at top of thigh) using a standard skin-fold caliper. A sample Physical Exam Form may be found in Appendix E.4.

Medical history. A review of each subjects medical history, including a history of cardiovascular, gastrointestinal, hepatic, endocrine, genitourinary, hematological, neurological, musculoskeletal, respiratory, immunological, neoplastic, dermatological, psychiatric, head and neck disorders, medications, allergies, HIV, surgical and traumatic history. A sample Medical History Form may be found in Appendix E.5. Personal habits were also assessed at screening. A sample Personal Habits Questionnaire may be found in Appendix E.6.

Data Quality Assurance

Data collection processes and procedures were reviewed and validated to ensure completeness, accuracy, reliability, and consistency. The student investigator cooperated with the Principal Investigator for the periodic review of source documents to ensure the accuracy and completeness of the data capture system. Electronic CT image scanning consistency checks and manual review of the source documents were used to identify errors or inconsistencies.

Study Monitoring and Auditing

In order to ensure the accuracy, consistency, completeness, and reliability of the data as well as include an independent review of adverse events, a Data Safety and Monitoring Board (DSMB) was assembled. This multidisciplinary group consisted of a biostatistician, radiologist, and physician who, collectively, had experience with the professional conduct and monitoring of clinical studies. The DSMB was responsible for safeguarding the interests of study participants, assessing the safety of the intervention during the study, and for monitoring the overall conduct of the clinical study. The DSMB team also provided advisory assistance to the study investigators. The investigators were responsible for promptly reviewing the DSMB

recommendations, to decide whether to continue or terminate the study, and to determine whether amendments to the protocol or changes in study conduct were required. At specific study intervals, the DSMB met with the student investigator to review any protocol changes, information pertaining to subject screening and withdrawal, eligibility violations (if any), baseline demographics of subjects, and any safety signals following dosing that required intervention. In addition to the DSMB oversight, the Medical Monitor was in constant communication with the investigators at regular intervals during the study.

Summary of Treatment Period Flow

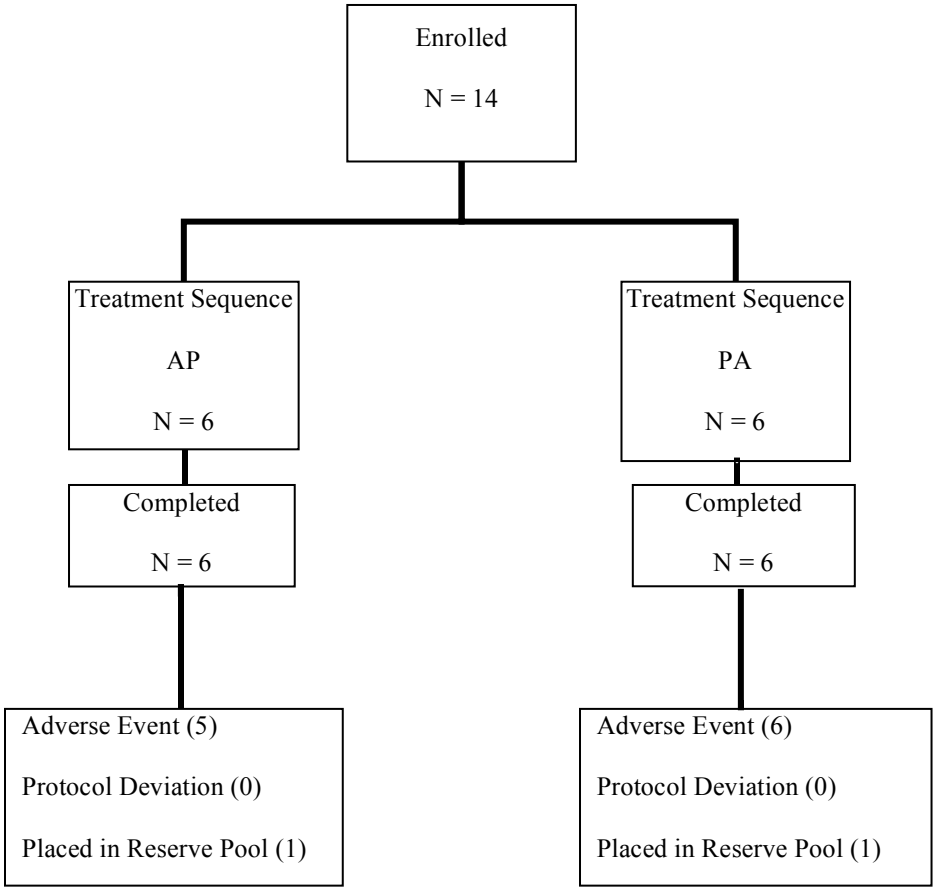
In summary, during each of the treatment periods, subjects reported to the CRSU to check-in and begin pre-study procedures which included confirmation of a 4-hour fast, obtaining vital signs within 60 minutes prior to dosing, review exclusion criteria, obtaining a urine pregnancy test (for females), and placement of a saline lock for blood draws. Subjects were transported to the Radiology Department from the CRSU 30 minutes prior to their scheduled scan time. CT scanning, blood draws, and urine collection occurred according to the sampling schedule and were tracked by the study flowsheets as found in Appendix E.1. Following the final plasma draw, after an overnight stay, subjects were placed on "pass" and allowed to go home or to class with urine containers for collection of urine for the 8-24 hour collection sample point. They then returned the urine at 24 hours where they had their vital signs and injection site assessed followed by discharge procedures.

Results

Disposition of Subjects

Subject disposition is presented in Figure 22. A review of the Participant Screening Log indicates that 19 volunteers underwent screening visits. Five subjects failed screening, either

because they withdrew consent or due to medical history, BMI, abnormal clinical laboratory tests, abnormal vital signs, abnormal ECGs, or other. Fourteen subjects were originally enrolled (randomized), 2 subjects were kept as reserves for the study, and 12 subjects completed the study. Eleven adverse events were reported. All were mild and resolved spontaneously as described in the Safety Evaluation section below.



N = number of subjects; A= Auto-injector; P = Pre-filled Syringe

Figure 22: Disposition of Subjects

Demographic and Other Baseline Characteristics

Demographic characteristics. Demographic variables are summarized by treatment sequence for the interim and post-interim subject populations in Tables 6 and 7, respectively. Descriptive statistics for the continuous and categorical variables by treatment sequence for all enrolled study subjects combined may be found in Appendices H.1 and H.2, respectively. Subjects in each treatment sequence were well matched for most all continuous and categorical variables with the exception of gender. Because of early enrollment challenges and the timing associated with scheduling the first two study subjects, the interim subject population included two males. This resulted in 60% of the subjects being female in the post-interim subject population. While there were slight differences between the treatment sequences in race, these differences were not expected to affect the study results or analyses.

Table 6: Summary of Demographic Variables by Treatment Sequence (Interim Analysis Population)

	Treatment Sequence		
Demographic Variable	**AP N=1**	**PA N=1**	**Overall N=2**
Continuous Variables: mean (SD)			
Age (years)	44	47	45.5 (1.73)
Height (cm)	177.5	186.0	181.75 (4.91)
Weight (kg)	79.2	90.0	84.6 (6.24)
Body mass index (kg/m²)	25.13	26.01	25.57 (0.51)
Thigh circumference (cm)	57.5	57.5	57.5 (0)
Categorical Variables: n (%)			
Race: Black or African American	1 (100.0)	1 (100.0)	2 (100.0)
Race: Asian	0 (0)	0 (0)	0 (0)
Race: White	0 (0)	0 (0)	0 (0)
Race: American Indian or Alaska Native	0 (0)	0 (0)	0 (0)
Gender: Female	0 (0)	0 (0)	0 (0)
Gender: Male	1 (100.0)	1 (100.0)	2 (100)

Table 7: Summary of Demographics Variables by Treatment Sequence (Post-Interim Analysis Population)

Demographic Variable	Treatment Sequence AP N=5	PA N=5	Overall N=10
Continuous Variables: mean (SD)			
Age (years)	23.8 (1.03)	30.2 (12.15)	27 (8.81)
Height (cm)	174.36 (8.59)	170.52 (10.79)	172.44 (9.63)
Weight (kg)	68.04 (7.51)	64.08 (9.26)	66.06 (8.41)
Body mass index (kg/m²)	22.35 (1.52)	21.90 (0.64)	22.13 (1.18)
Thigh circumference (cm)	45.16 (12.74)	50.56 (1.54)	47.86 (9.50)
Categorical Variables: n (%)			
Race: Black or African American	1 (20)	1 (20.0)	2 (20.0)
Race: Asian	1 (20.0)	0 (0)	1 (10.0)
Race: White	3 (60.0)	4 (80.0)	7 (70.0)
Race: American Indian or Alaska Native	0 (0)	0 (0)	0 (0)
Gender: Female	3 (60.0)	3 (60.0)	6 (60.0)
Gender: Male	2 (40.0)	2 (40.0)	4 (40.0)

Other baseline characteristics. None of the subjects enrolled had a clinically significant medical history or findings on screening physical examinations that would exclude them from participation in the study.

Interim Analysis

The first two subjects, (Subject # 306 and 307) underwent full study protocols as originally approved by the IRB, VCU Center for Clinical and Translation Research Protocol Review Committee, and Radiation Safety Committee. Both were African-American males, ages 44 and 47, respectively. These subjects received 150mg of iohexol 300 (0.5 mL at 300 mgI/mL) using the pre-filled syringe and auto-injector. Blood was drawn at pre-dose, 10, 20, 30, 40, 50 minutes and 1, 2, 3, 5, and 7 hrs post-dose. Urine samples were collected at pre-dose, 0-2, 2-3, 3-4, 4-6, 6-8, and 8-24 hours post-dose intervals. CT scans occurred at baseline, 0.5, 5, 10, 15 and 20 minutes.

Systemic exposure interim-analysis results. Plasma concentration data by sampling time point may be found in Table 8. Urine concentration by sampling time point may be found in Table 9 and the resultant urine concentration-time profile is shown in Figure 24. Each subject had quantifiable data with either the pre-filled syringe (subject 306) or auto-injector (subject 307); however, as depicted in the below Table 8 and as shown in the plasma concentration-time profile located in Figure 23, the systemic exposure data obtained was below the limit of quantitation for the majority of early plasma sampling time points, preventing further non-compartmental analysis from being conducted with the plasma data. Based upon this information, the student investigator in consultation with the principle investigator, DSMB and bioanalytical team, made the decision to move to the 300 mg dose and to adjust the plasma sampling schedule in order to improve the likelihood for measureable values with the remaining subjects.

Table 8: Plasma Concentrations Obtained for Interim Analysis (First Two Subjects)

Time (Minutes)	306-auto (ug/mL)	307-pfs (ug/mL)	306-pfs (ug/mL)	307-auto (ug/mL)
0	BLQ	BLQ	BLQ	BLQ
3	BLQ	BLQ	BLQ	BLQ
7	BLQ	BLQ	BLQ	BLQ
12	BLQ	BLQ	BLQ	BLQ
20	BLQ	BLQ	BLQ	BLQ
40	BLQ	BLQ	5.4780	BLQ
60	BLQ	3.0440	8.0500	3.5520
120	BLQ	3.1050	13.0870	11.0940
240	BLQ	BLQ	10.2110	10.6780
360	BLQ	BLQ	7.8890	9.5520
480	BLQ	BLQ	4.7700	6.5990

Table 9: Urine Concentration Obtained for Interim Analysis (First Two Subjects)

Time (Hours)	306-auto (ug/mL)	307-pfs (ug/mL)	306-pfs (ug/mL)	307-auto (ug/mL)
0	0	0	0	0
2	BLQ	69.66	315.75	289.50
4	381.34	121.07	1203.61	624.30
6	341.51	379.11	1713.31	1421.97
24	76.37	72.49	946.89	1395.40

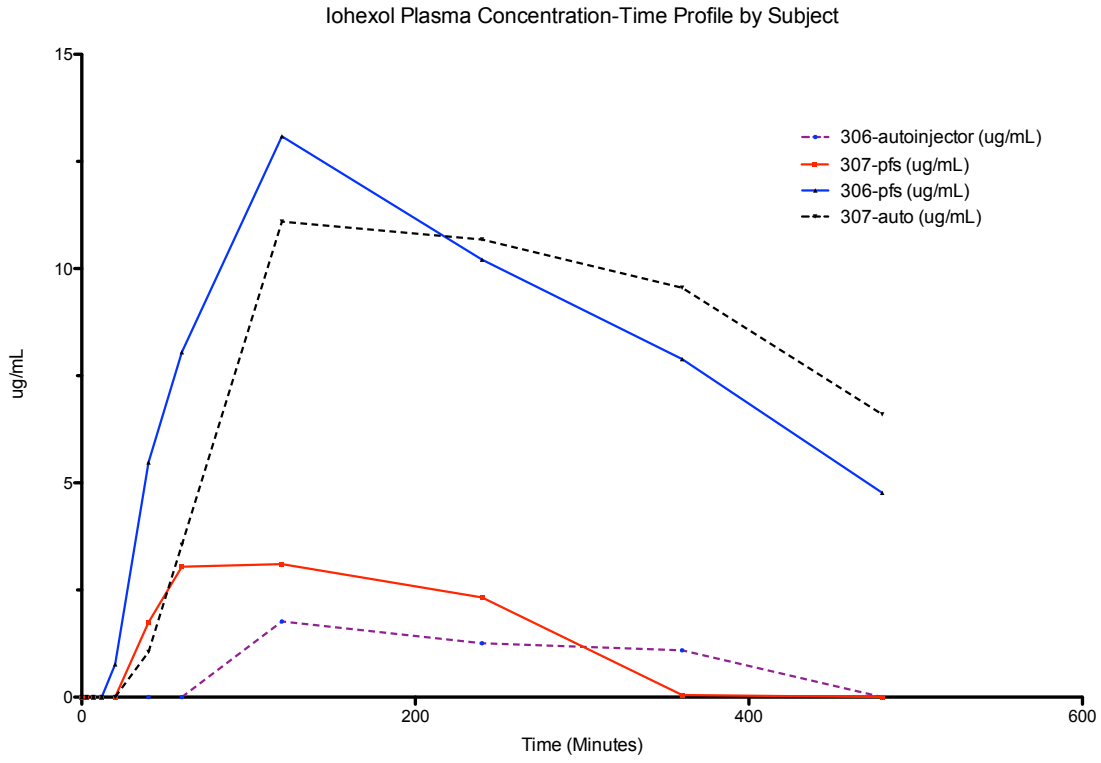


Figure 23: Plasma Concentration – Time Profile for Interim Analysis (First Two Subjects)

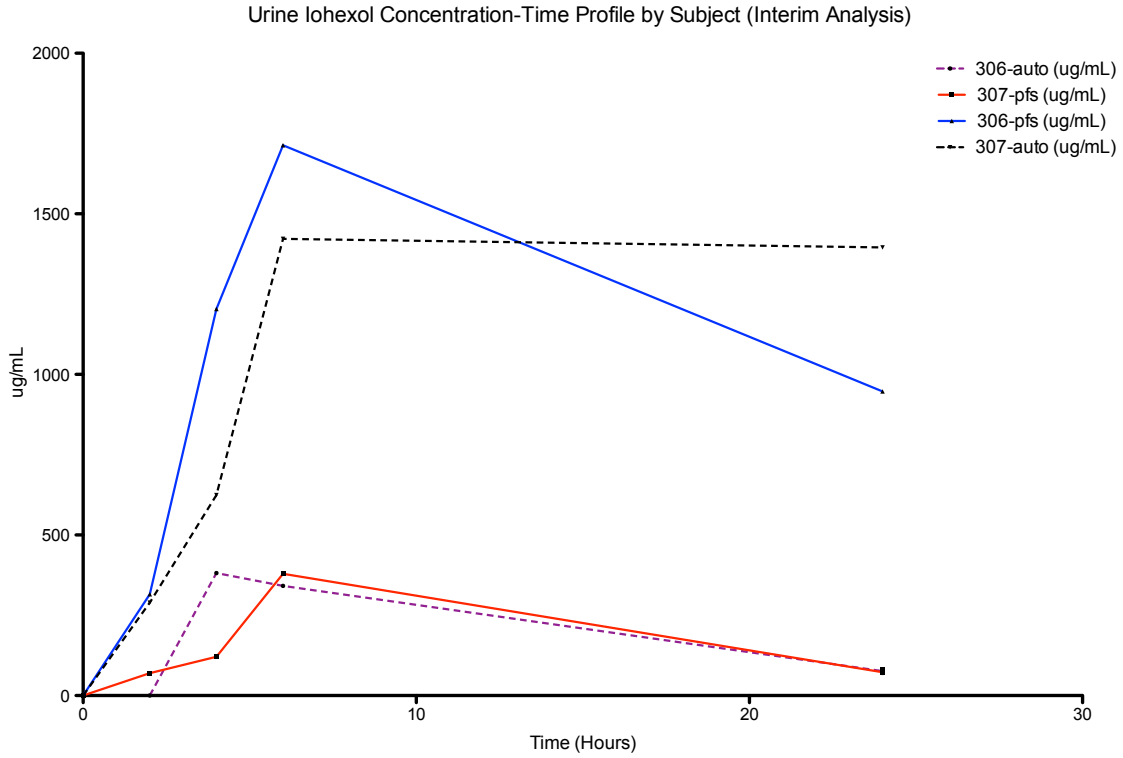


Figure 24: Urine Concentration – Time Profile for Interim Analysis (First Two Subjects)

Computed Tomography Interim-Analysis Scanning Results

Subject Computed Tomography scans were processed utilizing iSite Picture Archiving and Communications System (iSite PACS, Philips Koninklijke Electronics, ver. 3.6.52) software. Images were enhanced using the Laplacian Method to increase the visibility of edges in each image slice. Tissue discrimination was distinct for skin, subcutaneous tissue, quadriceps muscle groups, femur, and the bone marrow cavity.

Upon administration, iohexol could also be viewed in multiple image planes. For both interim subjects, the auto-injector reached beyond the subcutaneous tissue into the muscle. There was a substantial difference in the dispersion patterns obtained from 150 mg of iohexol delivery

using the PFS as compared to the auto-injector within each subject (Figures 25 - 28). Discrimination between intramuscular and subcutaneous injection was obtainable and quantitative measurements including location, pattern, maximum depth of dispersion (MDd), maximum width of dispersion (MWd), and depth at maximum width of dispersion (DMWd) could be assessed (Table 10). A sample depth at maximum width reading demonstrating the iSite PACS ruler function is provided in Figure 29. Additionally, radiodensity could be measured effectively using the software. Summary statistics are provided in Table 11 and an example of the radiodensity measurements over time are displayed in Figure 30.

Dispersion patterns were similar over time between subjects (Figures 31- 35). The quantitative measurements of dispersion seemed to suggest a consistent increase in the width of dispersion among all subjects over time, especially at the later time points (Table 10). Given this, it was recommended that the CT scanning time points should be changed from baseline, 0.5, 5, 10, 15 minutes to baseline, 0.5, 10 and 20 minutes for the remaining subjects.

Table 10: Dispersion Parameters for Interim Subject Population

Dispersion Parameter (mm)	306 – Auto	306 – PFS	307 – Auto	307 - PFS
Baseline Skin to Muscle Thickness	2.1	2.2	9.0	9.1
MDd (0.5 min)	19.8	14.2	13.1	16.3
MDd (2 min)	19.6	14.1	13.3	16.8
MDd (5 min)	19.0	14.0	13.5	16.5
MDd (10 min)	19.4	14.2	13.6	17.2
MDd (15 min)	19.4	13.5	12.8	17.7
MWd (0.5 min)	14.2	16.8	25.3	12.3
MWd (2 min)	14.4	17.3	25.5	12.5
MWd (5 min)	14.7	17.8	26.0	12.7
MWd (10 min)	15.1	18.4	26.6	12.9
MWd (15 min)	16.1	19.5	26.9	13.8
DMWd (0.5 min)	17.7	10.9	15.3	15.0
DMWd (2 min)	18.0	10.8	15.5	14.9
DMWd (5 min)	18.0	11.1	16.2	15.3
DMWd (10 min)	18.2	11.7	15.8	16.2
DMWd (15 min)	18.5	12.0	15.6	16.9

Table 11: Radiodensity Parameters for Interim Subject Population

Radiodensity Parameter (HU)	306 – Auto	306 – PFS	307 – Auto	307 - PFS
Radiodensity at 0.5 min	2821.57	2815.81	2927.72	2733.72
Radiodensity at 2 min	2648.82	2755.25	3071.00	2726.35
Radiodensity at 5 min	2610.03	2746.60	3068.09	2747.90
Radiodensity at 10 min	2601.07	2466.42	2873.33	2192.95
Radiodensity at 15 min	2420.84	2150.62	2529.32	1739.53

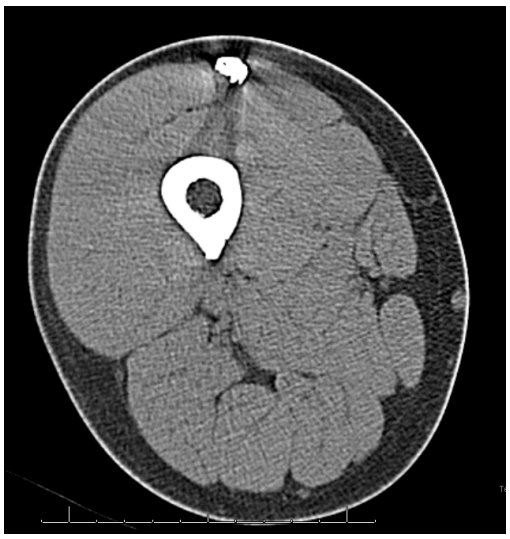


Figure 25: Subject 307 Iohexol by PFS into Thigh at 10 min (Axial Plane)

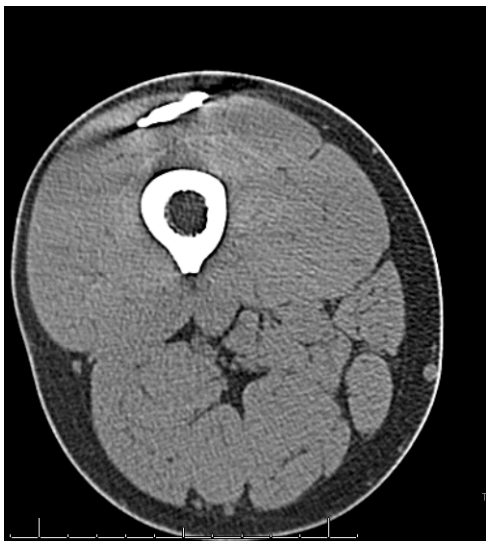


Figure 26: Subject 307 Iohexol by Auto-injector into Thigh at 10 min (Axial Plane)

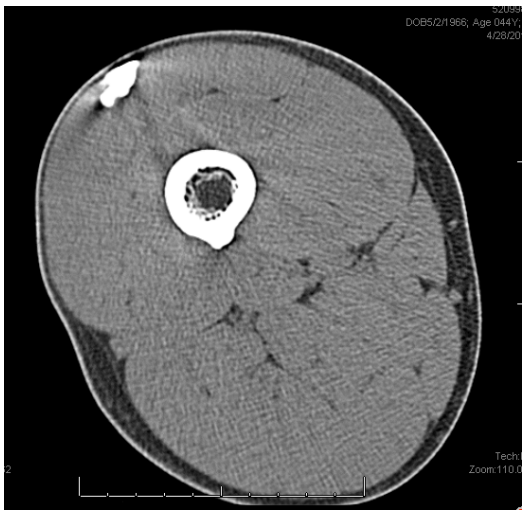


Figure 27: Subject 306 Iohexol by PFS into Thigh at 10 min (Axial Plane)

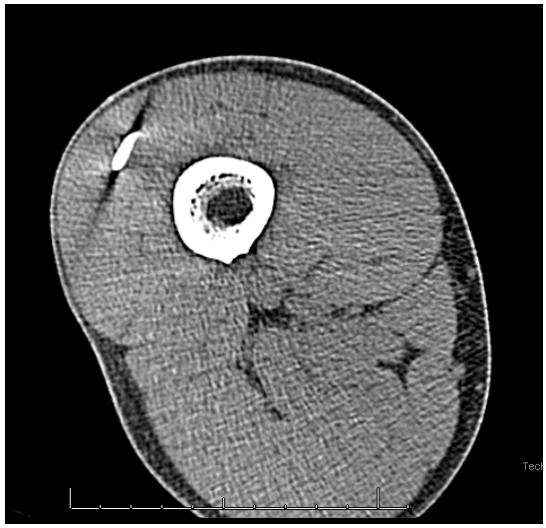


Figure 28: Subject 306 Iohexol by Auto-injector into Thigh at 10 min (Axial Plane)

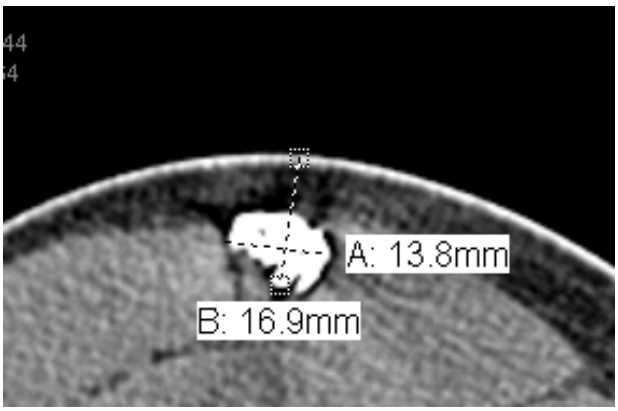


Figure 29: Example of DMWd Measurement using Ruler Tool in iSite PACs

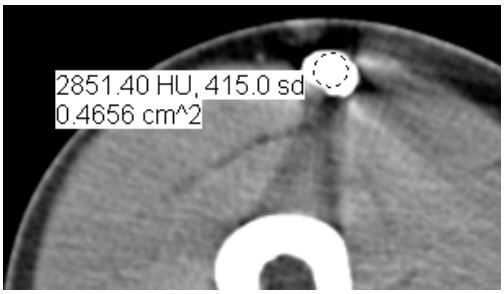
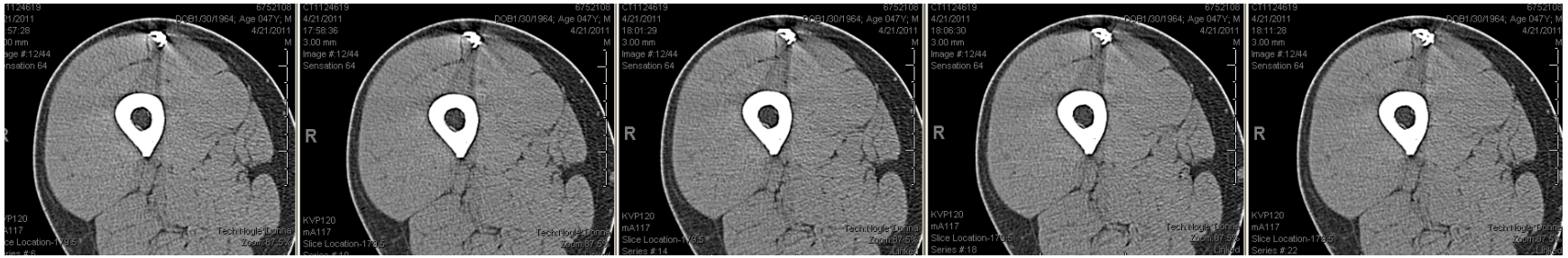
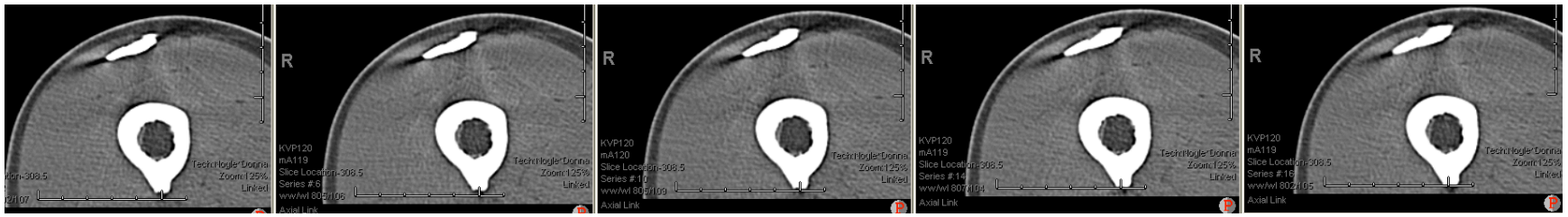


Figure 30: Example of Radiodensity Measurement using iSite PACs



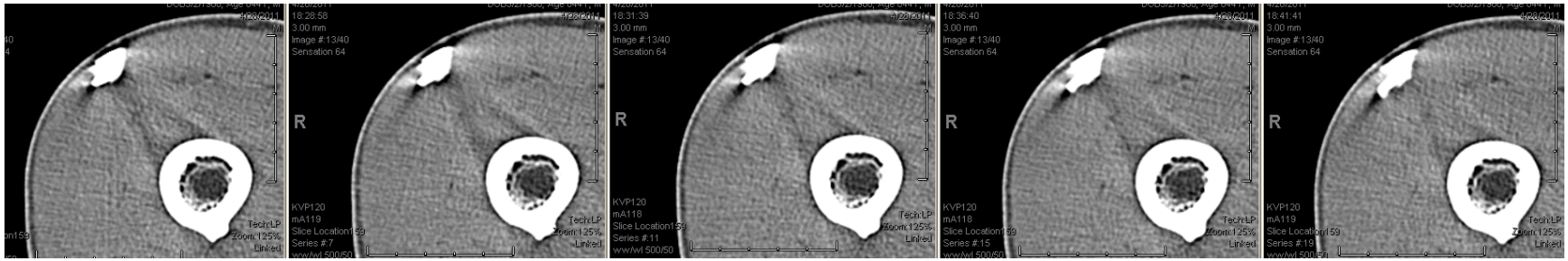
From Left to Right: 0.5min, 2min, 5min, 10min and 15min CT scans showing very little change in dispersion pattern

Figure 31: Subject 307 CT Scan - PFS Iohexol Dispersion in the Vastus Region of the Thigh over Time (Axial Plane)



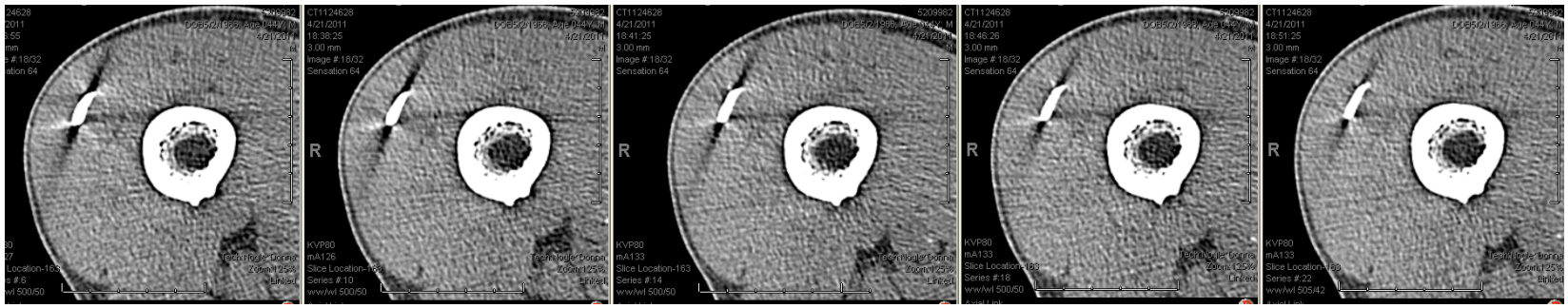
From Left to Right: 0.5min, 2min, 5min, 10min and 15min CT scans showing very little change in dispersion pattern

Figure 32: Subject 307 CT Scan - Auto-injector Iohexol Dispersion in the Vastus Region of the Thigh over Time (Axial Plane)



From Left to Right: 0.5min, 2min, 5min, 10min and 15min CT scans showing very little change in dispersion pattern

Figure 33: Subject 306 CT Scan - PFS Iohexol Dispersion in the Vastus Region of the Thigh over Time



From Left to Right: 0.5min, 2min, 5min, 10min and 15min CT scans showing very little change in dispersion pattern

Figure 34: Subject 306 CT Scan - Auto-injector Iohexol Dispersion in the Vastus Region of the Thigh over Time

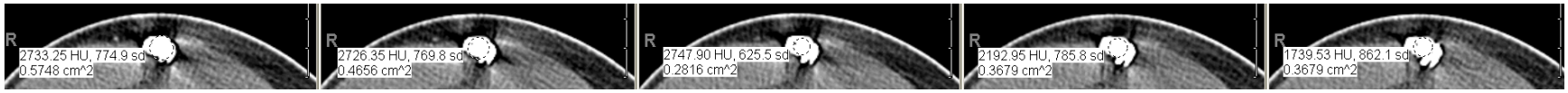


Figure 35: Representative Radiodensity Changes over Time (Axial Plane)

Interim Safety Results

The injection of iohexol by both delivery systems was well tolerated. No subjects experienced a single adverse event in either treatment period.

Interim Analysis Conclusions

The administration of iohexol (150 mgI/mL) in the first two subjects resulted in plasma and urine concentrations that were lower than anticipated, especially at the early sampling time points. However, the ability to obtain measureable iohexol concentrations to complete a sufficient non-compartmental analysis with the higher, 300 mgI/mL dose, and a new sampling schedule was deemed to be achievable by the study team. Computed tomography scanning resulted in images that provided excellent discrimination between intramuscular and subcutaneous injections and the ability to measure the desired quantitative parameters, including both dispersion and radiodensity measurements. Iohexol administration by either delivery system was well tolerated with no reported adverse events. Therefore, given the data obtained from this interim analysis along with the aforementioned proposed protocol changes, the investigators chose to proceed with the remaining 10 subjects.

Systemic Exposure Evaluation for Post-Interim Subject Population

Data sets analyzed. Twelve subjects were initially enrolled into the study. As previously discussed, the first two subjects finished both periods and were included in the interim analysis. Following this analysis, the protocol was modified to allow for a change in sampling time as well as an increase in the dose of iohexol administered (150 mg in 0.5mL to 300 mg in 1.0 mL). The systemic exposure population was defined as all subjects who had an evaluable plasma iohexol

concentration following protocol revision. Therefore, 10 subjects were included in the remaining data set.

Systemic exposure parameters. Concentration-time data of iohexol administered by the two delivery systems were examined using non-compartmental analysis assuming uniform weighting, extravascular input and linear interpolation with WinNonLin software (version 5.1; Pharsight Corporation, Mountain View, CA, USA). Missing concentration data values after time zero up to the first quantifiable concentration were set to "0". Missing values between concentrations or at the end of the profile were set to "missing."

The terminal rate constant, lambda z (λz), was determined from the slope of the terminal log-linear portion of the plasma-concentration-time curve, and the terminal half-life ($T_{1/2}$) was calculated as ln(2)/λz. Lambda z (λz) was calculated using at least three points, generally optimizing the correlation coefficient and *r*-squared (r^2) measure, while attempting to avoid the use of C_{max}. In some instances this was not possible. The goodness of fit statistic for the terminal elimination phase, with and without adjusting for the number of points used in the estimation of λz (r^2 and adjusted r^2, respectively) and the percentage of $AUC_{0-\infty}$ that was due to extrapolation from T_{last} to infinity was assessed to determine the $T_{1/2}$ and λz values. Subjects 309, 311, 312, 315, and 317 for the auto-injector and 304, 311, 314, 315, and 317 for the pre-filled syringe treatment groups were included in the analysis of $T_{1/2}$ and λz (See Table 12 for summary statistics). Details regarding the range of data points selected for the λz analysis by subject may be found in Appendix I.

Maximum plasma and urine concentrations (C_{max}) and time to maximum concentration

(T_{max}) were determined by direct observation of the data. The area under the concentration-time curve to the last non-zero plasma concentration that was above the lower limit of quantification was calculated as AUC_{0-t}. The area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + (C_{last}/\lambda z)$. To assess early exposure of iohexol after administration, partial area under the concentration-time curve was determined at various early exposure time points ($AUC_{partial} = AUC_{0-30}$, AUC_{0-40}, AUC_{0-60}, and AUC_{0-Tmax}). Means and standard deviations for the parameters were also obtained using the descriptive statistics tool in WinNonlin version 5.1. Further analysis using linear mixed-effect modeling was conducted using JMP Software (version 8.0; SAS Institute, Inc., Cary, NC, USA).

Systemic exposure analysis and results. Mean (± SD) observed Iohexol plasma concentration-time data by treatment are displayed graphically in Figure 36 (linear scale) and Figure 37 (semi-log scale). Observed iohexol plasma pharmacokinetic parameters are summarized descriptively in Table 12. Mean (± SD) observed Iohexol urine concentration-time data by treatment are displayed graphically in Figure 38 (linear scale) and Figure 39 (semi-log scale). Observed iohexol urine exposure parameters are summarized descriptively in Table 13. Individual subject plasma concentration-time profiles, including the graphical display of λz and associated goodness of fit measurements, may be found in Appendix J. The non-compartmental analyses output files may be found in Appendix K.

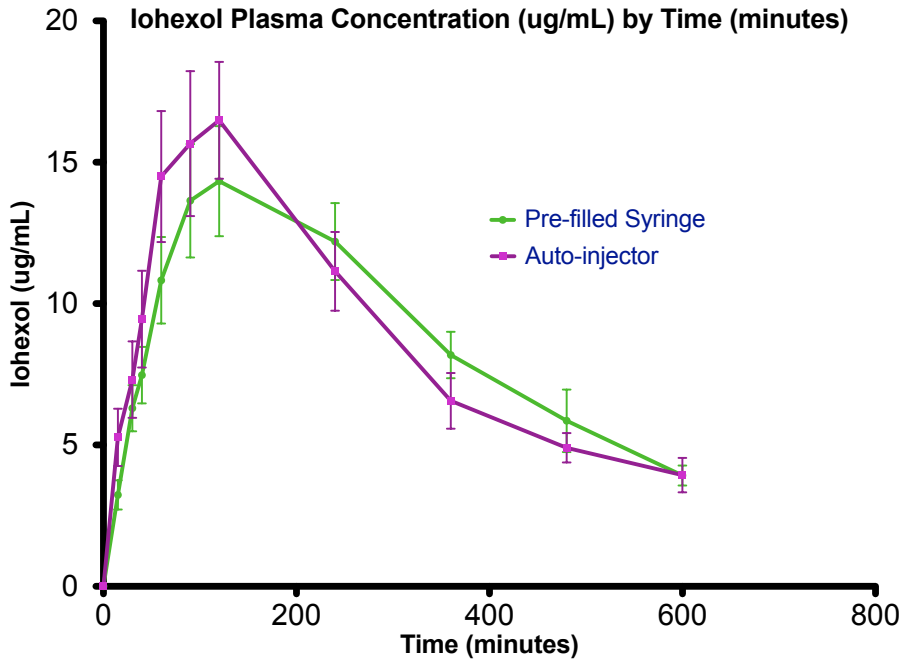


Figure 36: Mean ± SD Plasma Iohexol Concentration – Time Profiles by Delivery System (Observed) – Linear Scale

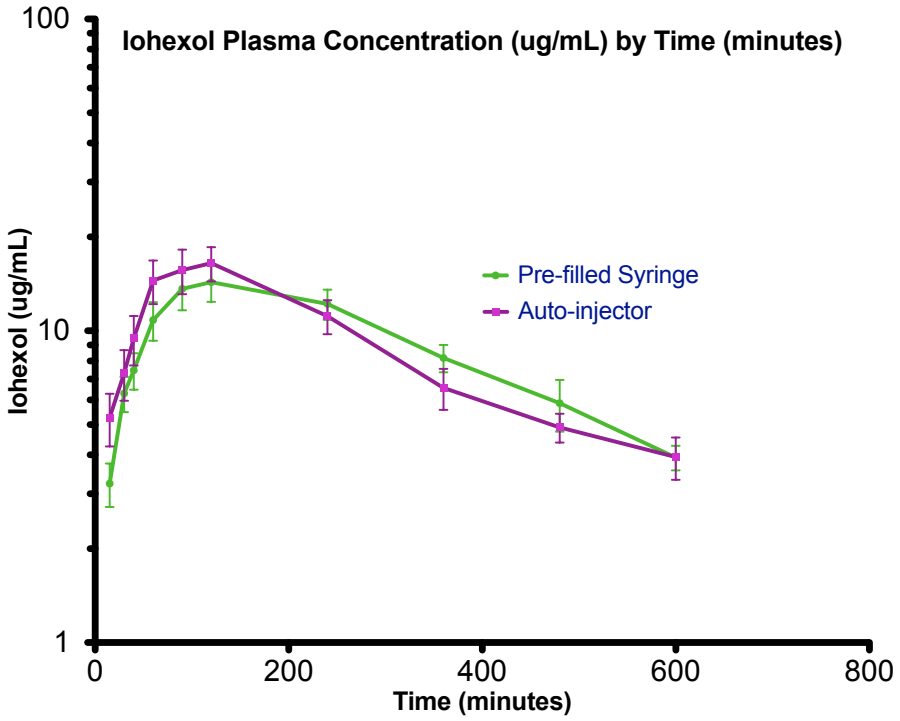


Figure 37: Mean ± SD Plasma Iohexol Concentration – Time Profiles by Delivery System (Observed) – Semi-log Scale

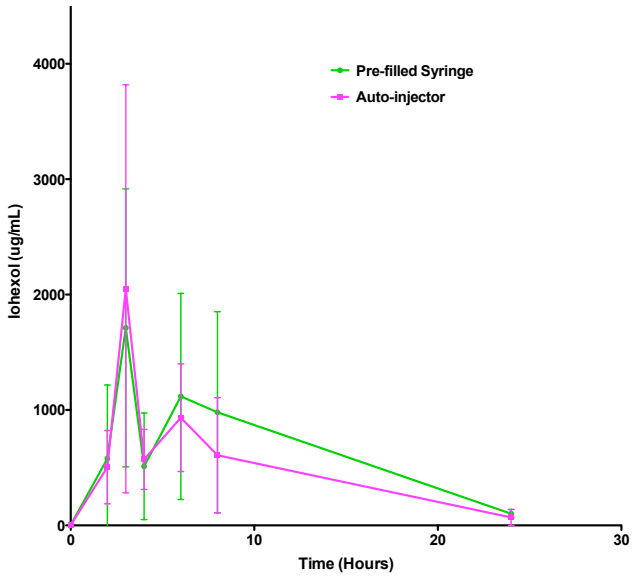


Figure 38: Mean ± SD Urine Iohexol Concentration – Time Profiles by Delivery System (Observed) – Linear Scale

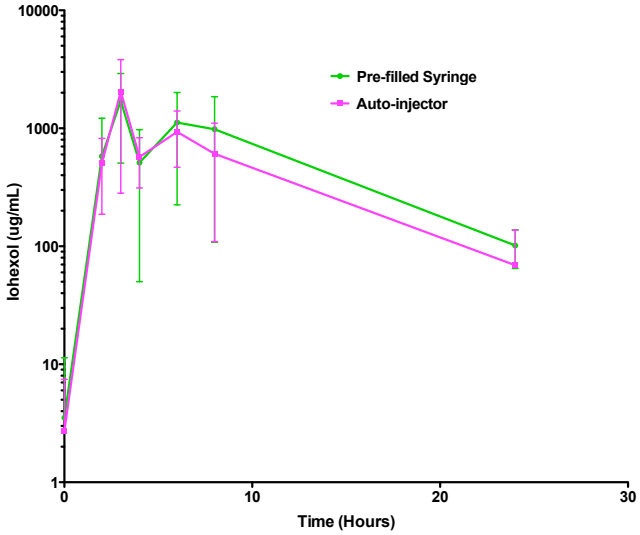


Figure 39: Mean ± SD Urine Iohexol Concentration – Time Profiles by Delivery System (Observed) – Semi-log Scale

Table 12: Summary of Iohexol Plasma Systemic Exposure Parameters

Treatment Group	Statistic	C_{max} (µg/mL)	T_{max} (min)	$T_{½}$ (min)*	Lambda z (1/min)*	AUC_{0-t} (µg·min/mL)	$AUC_{0-\infty}$ (µg·min/mL)
Pre-filled Syringe	**n**	10	10	5	5	10	9
	Mean	16.395	147.0	139.132	0.00532	4852.788	6365.609
	SD	5.087	65.5	38.944	0.00150	1360.513	1463.046
	Median	15.560	120.0	127.840	0.00540	5065.710	6159.300
	Min	9.170	90.0	92.820	0.00360	2493.170	3477.390
	Max	26.370	240.0	192.510	0.00750	7181.580	8744.200
	CV (%)	31.03	44.56	27.99	28.18	28.04	22.98
Auto-Injector	**N**	10	10	5	5	10	9
	Mean	17.037	120.0	142.954	0.00486	4555.656	5788.618
	SD	6.871	44.7	8.858	0.000321	2127.979	2338.269
	Median	15.950	120.0	141.930	0.00490	4804.670	5959.100
	Min	7.267	90.0	130.440	0.00450	2095.780	2715.540
	Max	29.800	240.0	152.800	0.00530	7980.810	8560.270
	CV (%)	40.33	37.27	6.20	6.60	46.71	40.39

* Note: Analysis completed with subset of population

Table 13: Summary of Iohexol Urine Systemic Exposure Parameters

Treatment Group	Statistic	C_{max} (µg/mL)	T_{max} (hrs)	AUC_{0-t} (µg·hr/mL)
Pre-filled Syringe	**n**	10	10	10
	Mean	2122.4	4.7	14895.12
	SD	1057.5	2.5	9352.06
	Median	1902.0	3.0	12581.50
	Min	714.8	2.0	5597.65
	Max	4386.0	8.0	34357.20
	CV (%)	49.83	53.12	62.79
Auto-injector	**N**	10	10	10
	Mean	2305.7	4.3	11315.09
	SD	1564.2	2.2	4906.74
	Median	1761.5	3.0	11147.70
	Min	717.3	3.0	3227.75
	Max	5714.0	8.0	19347.10
	CV (%)	67.84	50.30	43.36

Iohexol plasma peak (C_{max}) and total (AUC_{0-t}) exposure were similar whether administered by pre-filled syringe or auto-injector. Mean C_{max} values were approximately 16.4 µg/mL and 17.0 µg/mL for the PFS and auto-injector groups, respectively. Mean AUC_{0-t} values were 4852.79 and 4555.66 µg·min/mL for the PFS and auto-injector groups, respectively (Table 12). The between-subject variability in these parameters was high (CV% ranged from approximately 28 to 47%). Iohexol peak plasma exposure took more time than anticipated; median T_{max} was 120 minutes and ranged from approximately 90 to 240 minutes for both products. Plasma T_{max} values were highly variable as suggested by the broad range of observed values and by CV% values of 45% and 37% for the pre-filled syringe and auto-injector products, respectively. Mean plasma $T_{1/2}$ was similar in both treatment groups occurring at approximately 140 minutes (139 minutes for the PFS and 143 minutes for the auto-injector). Lambda z (λz) was also similar for both treatments (0.005 hr^{-1}) suggesting a similar rate of elimination from the systemic circulation. Finally, because more than 20% of the plasma $AUC_{0-\infty}$ estimates were extrapolated for many of the subjects in both treatment groups, no conclusions could be drawn associated with this parameter.

Iohexol urine peak (C_{max}) and total (AUC_{0-t}) exposure as well as time to maximum exposure (T_{max}) were similar whether administered by pre-filled syringe or auto-injector and highly variable (the between-subject variability was over 40% for all parameters) (See Table 13). The urine concentration-time profiles were similar by treatment group exhibiting two peaks that were approximately 3 hours apart.

Mixed model assessment of systemic exposure parameters. Linear mixed-effects models were used to test for differences in mean plasma C_{max}, T_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ between the Auto-Injector and PFS treatment groupings after controlling for period and sequence effects as well as the covariates of age, sex, and thigh circumference. The models included a random subject effect to account for within subject variations. Additionally, the models included fixed effects for treatment, period, and sequence as well as the aforementioned covariates. Each of these covariates were included in the model in order to determine what effects each, or any may have on the response parameters (C_{max}, T_{max}, AUC_{0-t}, and $AUC0_{0-\infty}$). Using these models, the mean responses (C_{max}, T_{max}, AUC_{0-t}, and $AUC0_{0-\infty}$) were estimated for each group (Table 14) and the differences in the means were compared statistically between the groups using a significance level of $\alpha = 0.05$ (Table 15).

Plasma C_{max} and T_{max}. The mixed-effects model accounted for 94.9% (r^2 adjusted = 91.0%) of the variations in C_{max} and 52.6% (r^2 adjusted = 18.2 %) of the variations in T_{max}. There was no evidence of a significant treatment effect on either C_{max} ($p = 0.5021$) or T_{max} ($p = 0.2914$). There were no significant effects of any of the covariates on either C_{max} or T_{max} with the exception of a significant period effect on C_{max} ($p = 0.0025$), with mean C_{max} significantly lower in the first period than the second (12.52 µg/mL vs. 16.49 µg/mL). It is unclear what may have led to this difference. One possibility could be subjects having less anxiety during the second period; therefore, there may have been less endogenous, epinephrine-induced vasoconstriction that could slow absorption in the second period leading to greater overall absorption. The least

squares mean C_{max} and T_{max} estimated from the model are summarized by treatment group in Table 14 and the difference between groups is summarized in Table 15.

Extent of exposure (AUC_{0-t} and $AUC_{0-\infty}$). The mixed-effects model accounted for 87.8% (r^2 adjusted = 79.0%) of the variations in AUC_{0-t} and 71.3% (r^2 adjusted = 45.8%) of the variations in $AUC_{0-\infty}$. There was no evidence of significant treatment effects (AUC_{0-t}, p = 0.4799; $AUC_{0-\infty}$, p = 0.2932) or other model covariates on either AUC_{0-t} or $AUC_{0-\infty}$ with the exception of a significant period effect on AUC_{0-t} (p = 0.0010). The mean AUC was significantly lower in the first period than the second (2992.25 µg·min/mL vs. 4959.03 µg·min/mL for AUC_{0-t}. There were no substantial changes in the results when comparing the untransformed data to the log transformed data. Mean AUC_{0-t} and $AUC_{0-\infty}$ are summarized by treatment group in Table 14 and the difference between groups is summarized in Table 15.

Table 14: (Adjusted) Mean Response Measures by Treatment Group

	Auto-injector			Pre-filled Syringe		
Response	**Mean**	**SE**	**95% CI**	**Mean**	**SE**	**95% CI**
C_{max} (µg/mL)	14.83	2.88	(5.92, 23.74)	14.19	2.88	(5.28, 23.10)
T_{max} (min)	126.27	27.90	(51.61, 200.92)	122.53	27.90	(78.61, 227.92)
AUC_{0-t} (µg·min/mL)	3792.07	721.94	(1673.24, 5910.90)	4089.20	721.94	(1970.37, 6208.03)
$AUC_{0-\infty}$ (µg·min/mL)	5273.56	750.32	(3410.27, 7136.84)	6085.31	746.67	(4173.44, 7997.17)

Table 15: (Adjusted) Mean Differences in Response Measures between Treatment Groups

Response	Difference	SE	95% CI
C_{max} (µg/mL)	0.64	0.91	(-1.47, 2.75)
T_{max} (min)	27.00	23.91	(-28.13, 82.13)
AUC_{0-t} (µg·min/mL)	297.13	401.04	(-627.66, 1221.93)
$AUC_{0-\infty}$ (µg·min/mL)	811.75	715.06	(-874.35, 2497.85)

† Indicates statistically significant at $\alpha = 0.05$

Systemic exposure analysis of early exposure. In order to assess early plasma exposure parameters of iohexol, a partial AUC analysis was conducted at various time points (30, 40 and 60 minutes) as well as up to T_{max} for each treatment group. In addition, a calculation of C_{max}/AUC_{0-t} was conducted as a measurement of absorption rate. Although the rate of absorption (using C_{max}/AUC_{0-t}) was similar between treatment groups (approx. 0.004 min^{-1}) there was a difference in the extent of early systemic exposure, with the mean AUC difference between treatment groups increasing over time up until 60 minutes post-dose (Table 16). This difference was not apparent by the time the maximum plasma concentration, T_{max} ,was reached as mean AUC_{0-tmax} was similar for the PFS (1281.06 µg·min/mL) and auto-injector (1110.32 µg·min/mL) groups. Although the between-subject variability was high for these early exposure parameters up until 60 minutes post-dose (CV% ranging from approximately 55% to 82%), these results suggest the delivery system may have had an effect on the extent of early exposure to iohexol (see mixed-effects model analysis below).

The time prior to the time point corresponding to the first measurable (non-zero) concentration was calculated as T_{lag}. Similar T_{lag} data were obtained relative to the pre-filled syringe (mean 27.5 min) and auto-injector (22.5 min) products; however, the between-subject variability in these parameters was high (CV% ranged from approximately 59% to 82%) limiting

the ability to draw definitive comparative conclusions regarding the average time for removal from the extravascular tissue "compartment" to the systemic circulation.

Table 16: Summary of Iohexol Plasma Early Exposure Parameters

Treatment Group	Statistic	T_{lag} (min)	C_{max} /AUC_{0-t} (min^{-1})	$AUC_{(0-30min)}$ (µg·min/mL)	$AUC_{(0-40min)}$ (µg·min/mL)	$AUC_{(0-60min)}$ (µg·min/mL)	$AUC_{(0-Tmax)}$ (µg·min/mL)
Pre-filled Syringe	**n**	8	10	6	8	9	10
	Mean	27.5	0.00355	63.427	108.539	266.410	1281.058
	SD	16.3	0.00137	38.184	71.627	149.138	612.214
	Median	22.5	0.00302	46.895	114.250	295.080	1323.940
	Min	15.0	0.00237	23.850	16.200	49.340	584.360
	Max	60.0	0.00716	115.350	208.440	500.110	2475.950
	CV (%)	59.12	38.66	60.20	65.99	55.98	47.79
Auto-injector	**n**	6	10	9	9	9	10
	Mean	22.5	0.00405	89.986	173.801	413.271	1110.323
	SD	18.34	0.00135	73.939	118.359	236.291	371.503
	Median	15.0	0.00369	44.870	116.460	341.180	1109.200
	Min	15.0	0.00255	30.740	75.590	165.840	541.800
	Max	60.0	0.00718	226.430	395.530	865.830	1728.930
	CV (%)	81.65	33.29	82.17	68.10	57.18	33.46

Mixed model assessment of early exposure parameters. Mixed-effects models were also used to test for differences in untransformed and log transformed mean early exposure parameter data ($AUC_{0\text{-}30}$, $AUC_{0\text{-}40}$, $AUC_{0\text{-}60}$, and $AUC_{0\text{-}Tmax}$) between the Auto-Injector and PFS delivery systems after controlling for the covariates of period, sequence effects, age, sex, and thigh circumference. The models included a random subject effect to account for within subject variations and fixed effects for treatment, period, and sequence as well as the aforementioned covariates. Each of these covariates were included in the model in order to determine what effects each, or any may have on the response parameters ($AUC_{0\text{-}30}$, $AUC_{0\text{-}40}$, $AUC_{0\text{-}60}$, and $AUC_{0\text{-}Tmax}$) by treatment group. Using these models, the mean responses ($AUC_{0\text{-}30}$, $AUC_{0\text{-}40}$, $AUC_{0\text{-}60}$, and $AUC_{0\text{-}Tmax}$) were estimated for each group (Table 17) and the differences in the means were compared statistically between the groups using a significance level of $\alpha = 0.05$ (Table 18).

$AUC_{0\text{-}30}$, $AUC_{0\text{-}40}$, and $AUC_{0\text{-}60}$. The mixed-effects model accounted for the vast majority (greater than 93%) of the variations in $AUC_{0\text{-}30}$, $AUC_{0\text{-}40}$ and $AUC_{0\text{-}60}$ ($AUC_{0\text{-}30}$ r^2 adjusted = 83.7%, $AUC_{0\text{-}40}$ r^2 adjusted = 85.8%, $AUC_{0\text{-}60}$ r^2 adjusted = 92.0%). There was no evidence of a significant treatment effect on $AUC_{0\text{-}30}$ ($p = 0.0630$); however, there were significant treatment effects on $AUC_{0\text{-}40}$ and $AUC_{0\text{-}60}$ ($p = 0.0347$ and $p = 0.0113$, respectively). The mean response for the Auto-injector treatment group was significantly higher than for the PFS treatment group ($AUC_{0\text{-}40}$ difference = 73.27, 95% CI = 7.81, 138.73; $AUC_{0\text{-}60}$ difference = 126.63, 95% CI = 40.60, 212.66) (Table 18).

There were also significant period effects for each of these early exposure parameters with mean $AUC_{0\text{-}30}$, $AUC_{0\text{-}40}$, and $AUC_{0\text{-}60}$ significantly lower in the first period than the second ($p = 0.0144$, $p = 0.0167$, $p = 0.0049$, respectively). There were not significant effects of any of the other covariates on $AUC_{0\text{-}30}$, $AUC_{0\text{-}40}$, or $AUC_{0\text{-}60}$. There were no substantial changes in the results when comparing the untransformed data to the log transformed data.

$AUC_{0\text{-}Tmax}$. The mixed-effects model accounted for only 12.6% (r^2 adjusted = -0.51%) of the variations in $AUC_{0\text{-}Tmax}$. There was no evidence of significant treatment effects ($AUC_{0\text{-}Tmax}$ ($p = 0.5078$) or other model covariates on $AUC_{0\text{-}Tmax}$. There were no substantial changes in the results when comparing the untransformed data to the log transformed data.

Table 17: (Adjusted) Mean Response Measures by Treatment Group

	Auto-injector			Pre-filled Syringe		
Response	**Mean**	**SE**	**95% CI**	**Mean**	**SE**	**95% CI**
$AUC_{0\text{-}30}$ (µg·min/mL)	46.29	35.88	(-99.89, 192.48)	3.50	38.77	(-124.78, 131.79)
$AUC_{0\text{-}40}$ (µg·min/mL)	136.16	58.12	(-51.71, 324.03)	62.89	58.75	(-123.75, 249.53)
$AUC_{0\text{-}60}$ (µg·min/mL)	324.09	107.89	(-11.23, 659.41)	197.46	107.02	(-140.72, 535.64)
$AUC_{0\text{-}Tmax}$ (µg·min/mL)	898.42	196.55	(434.06, 1362.79)	1069.16	196.55	(604.80, 1533.52)

Table 18: (Adjusted) Mean Differences in Response Measures

Response	**Difference**	**SE**	**95% CI**	
$AUC_{0\text{-}30}$ (µg·min/mL))	42.79	16.87	(-3.68, 89.26)	
$AUC_{0\text{-}40}$ (µg·min/mL)	73.27	25.52	(7.81, 138.73)	†
$AUC_{0\text{-}60}$ (µg·min/mL)	126.63	35.24	(40.60, 212.66)	†
$AUC_{0\text{-}Tmax}$ (µg·min/mL)	170.74	246.30	(-397.23, 738.70)	

† Indicates statistically significant at $\alpha = 0.05$

Computed Tomography Scanning Analysis for Post-Interim Subject Population

Data sets analyzed. Twelve subjects were initially enrolled into the study. As discussed in the Computed Tomography (CT) Scanning Parameter Measurements section, following the interim analysis, the protocol was modified to allow for a change in computed tomography scanning time points from 0.5, 5, 10, 15 and 20 minutes for the first two interim analysis subjects to 0.5 10, and 20 minutes for the subsequent 10 subjects. The CT scanning population was defined as all subjects who had an evaluable computed tomography scan following protocol revision. Therefore, 10 subjects were included in the remaining CT scanning data set.

Computed tomography parameters. Subject Computed Tomography scans were processed utilizing iSite Picture Archiving and Communications System (iSite PACS, Philips Koninklijke Electronics, ver. 3.6.52) software. Images were enhanced using the Laplacian Method to increase the visibility of edges in each image slice. Determination of location of iohexol injection was made by direct CT scan observation. Measurements of maximum depth of dispersion (MDd) were completed utilizing a ruler tool in millimeter units by selecting the point on the skin surface (epidermal region) and measuring to the deepest detectable level of iohexol contrast media for a given axial image slice. Measurements of maximum width of dispersion (MWd) were completed utilizing the ruler tool by selecting the axial image slice with the widest tissue dispersion of iohexol. Finally, measurements of the depth at maximum width of dispersion (DMWd) were completed utilizing the ruler tool to measure the depth at the axial image slice with the widest tissue dispersion of iohexol at each time point by selecting the point on the skin

surface (epidermal region) and measuring to the deepest detectable level of iohexol contrast media.

Means and standard deviations for the parameters of total depth of dispersion, width of RCM dispersion, and depth of dispersion to maximum width were obtained using the descriptive statistics tool in JMP Software (version 8.0; SAS Institute, Inc., Cary, NC, USA). Further analysis using mixed-effect modeling was also conducted using the JMP Software.

In order to assess the rate and extent of disappearance or loss of Iohexol from the extravascular compartment over time, a radiodensity measurement was calculated utilizing the radiodensity tool iSite PACs software. Each image was assessed for radiodensity at the slice corresponding to the depth at maximum width of dispersion (DMWd) at each time point. Radiodensity was plotted over time and the rate of iohexol elimination from the extravascular tissue ($Loss_{Tiss}$) was estimated using the slope from the fit line calculated from the linear regression model for each individual subject utilizing the JMP software.

Computed tomography qualitative analysis. Tissue discrimination was distinct for skin, subcutaneous tissue, quadriceps muscle groups, femur, and the bone marrow cavity. Upon administration, iohexol could be viewed in multiple image planes (Figure 40). There was a notable difference in the dispersion pattern between male and female subjects, likely due to the underlying differences in subcutaneous tissue depth. Representative CT scan images from a male versus female subject using the auto-injector delivery system over time may be found in Figures 41 and 42.

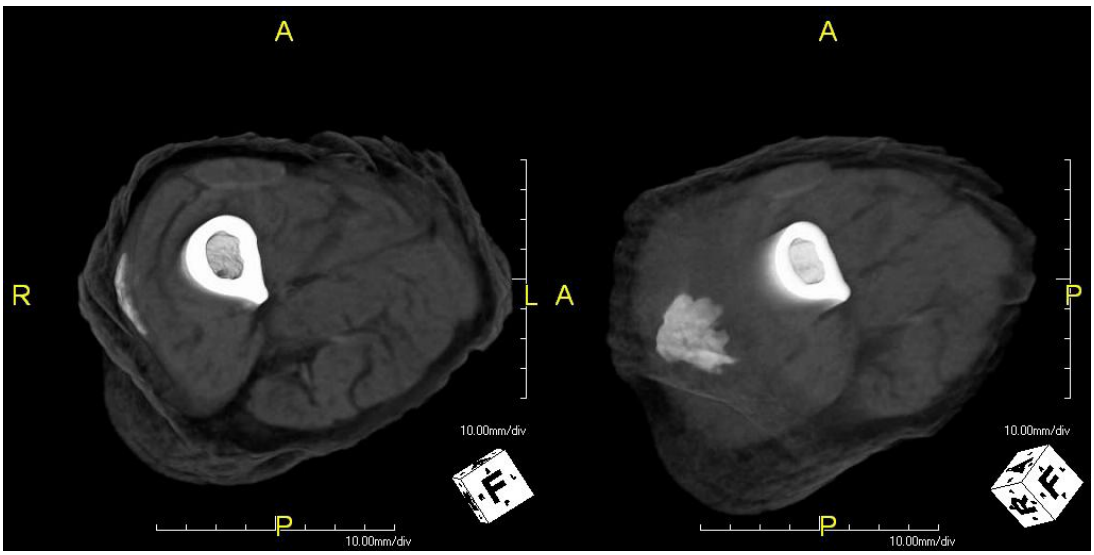
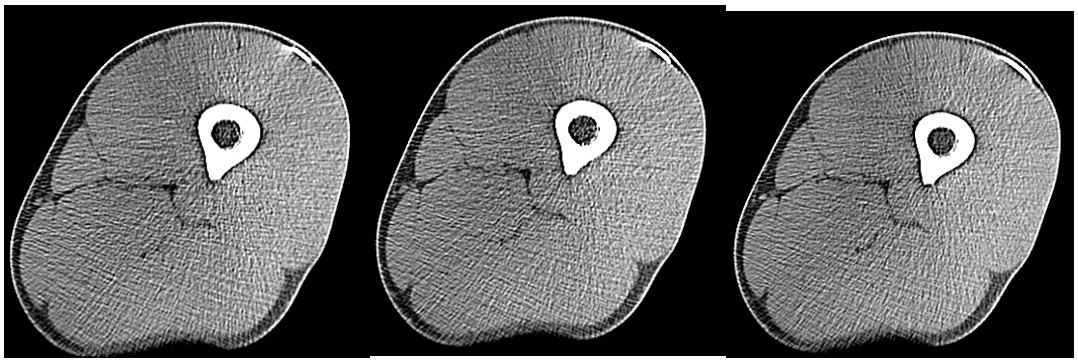
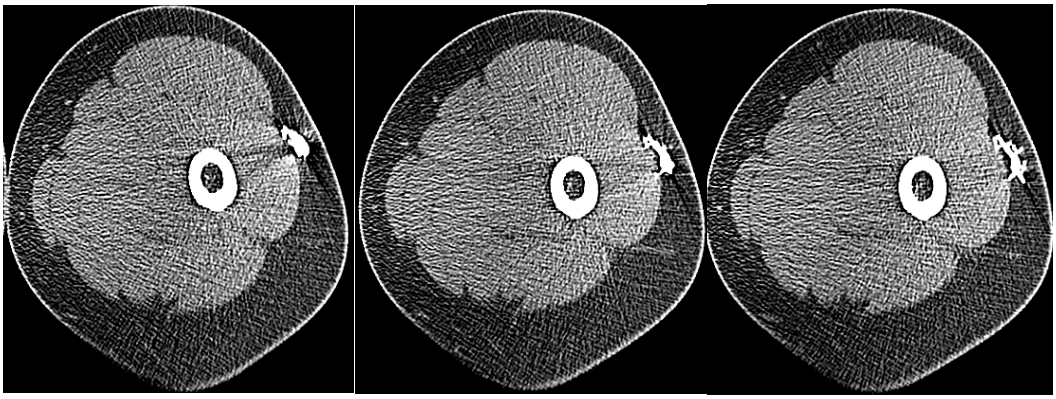


Figure 40: Iohexol Dispersion in Axial (Left) and Oblique (Right) Viewing Planes



From left to right: Iohexol dispersion patterns at 30 seconds, 10 minutes, and 20 minutes

Figure 41: Representative Male Subject Auto-Injector Dispersion Pattern over Time



From left to right: Iohexol dispersion patterns at 30 seconds, 10 minutes, and 20 minutes

Figure 42: Representative Female Subject Auto-injector Dispersion Pattern over Time

Additionally, 40% of the subjects had iohexol tissue dispersion that includes interstices penetrating the muscle (Figure 43) versus solely remaining in the subcutaneous tissue as intended (Figure 44).

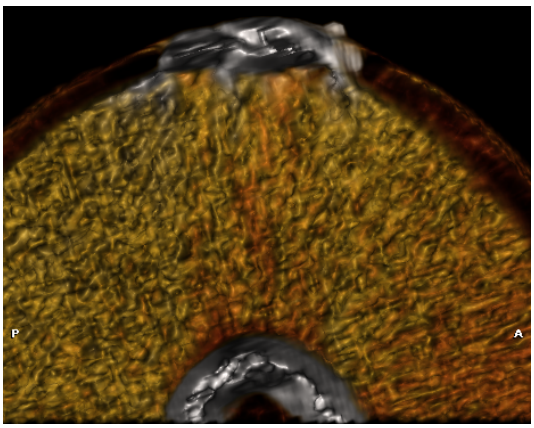


Figure 43: Iohexol Dispersed in the Subcutaneous and Intramuscular Tissue

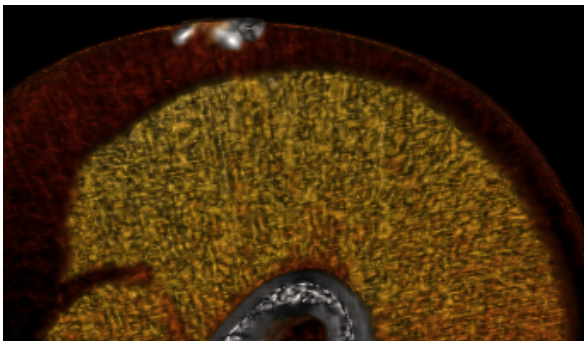


Figure 44: Iohexol Dispersed in the Subcutaneous Tissue

The images could be reconstructed in three dimensions using the imaging software which allowed for greater discrimination of the dispersion differences between the delivery systems over time. As seen in Figures 45 and 46, the auto-injector appeared to produce a more widely distributed dispersion pattern as compared with the pre-filled syringe alone.

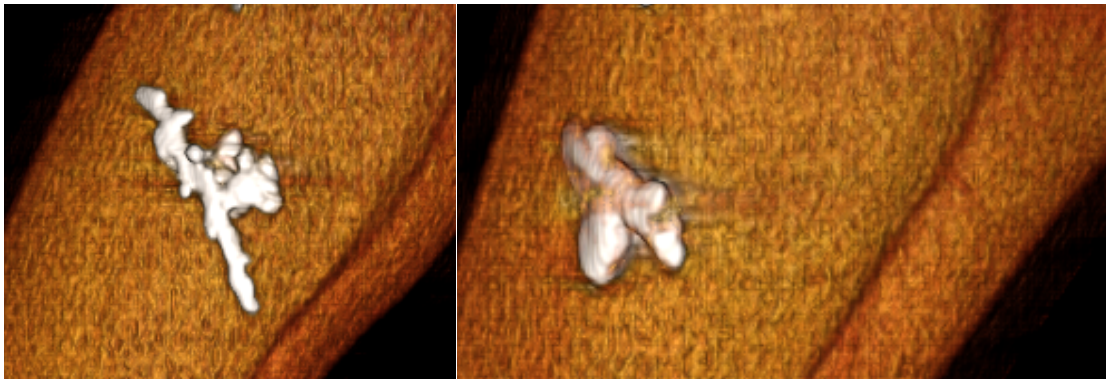


Figure 45: Representative Subject A - Dispersion Pattern by Auto-injector (Left) versus PFS at 10 minutes (Right)

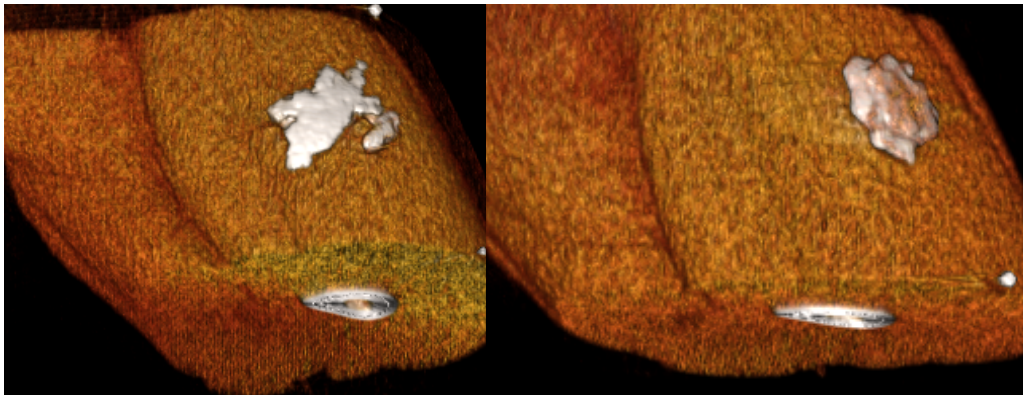
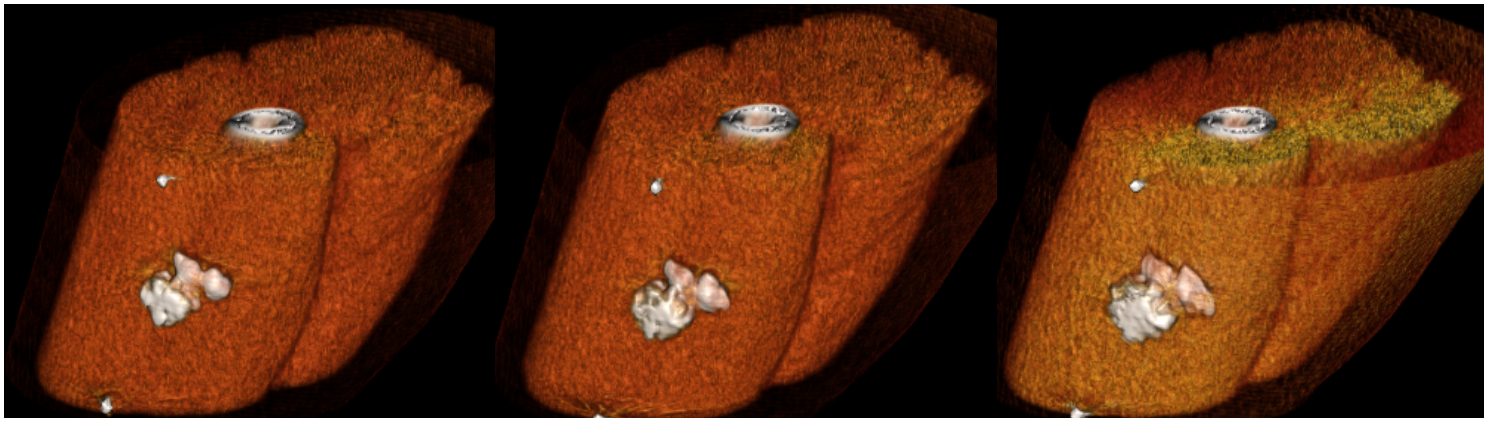


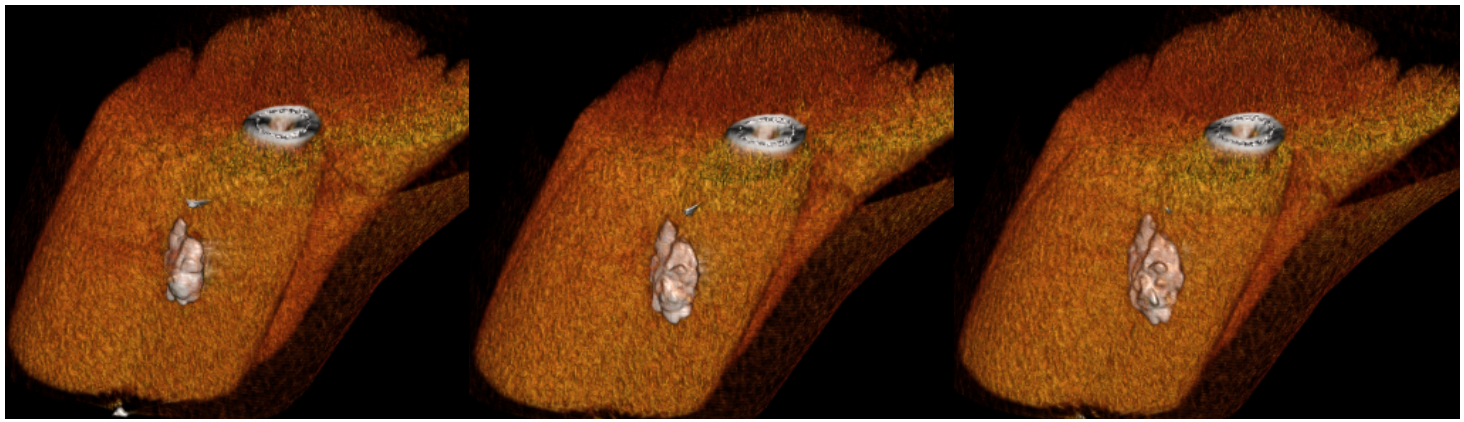
Figure 46: Representative Subject B - Dispersion Pattern by Auto-injector (Left) versus PFS at 10 minutes (Right)

Furthermore, the difference in dispersion patterns could be discriminated over time in the three dimensions. The auto-injector appeared to have visibly, a greater width of dispersion over time as compared to the pre-filled syringe delivery system. A representative subject scan demonstrating this is shown in Figures 47 and 48.



From left to right: Iohexol dispersion patterns at 30 seconds, 10 minutes, and 20 minutes

Figure 47: Representative Iohexol Dispersion by Auto-injector Delivery System over Time



From left to right: Iohexol dispersion patterns at 30 seconds, 10 minutes, and 20 minutes

Figure 48: Representative Iohexol Dispersion by PFS Delivery System over Time

Computed tomography quantitative analysis and results. Observed iohexol dispersion and radiodensity parameters are summarized descriptively in Tables 19 and 20, respectively. Mean iohexol dispersion over time was similar for all parameters between the two treatment groups. Additionally, the iohexol dispersion measurements were similar at each scanning time point whether by auto-injector or PFS with the exception of the Maximum Width of Dispersion (MWd) parameters. The MWd increased over time for both treatments (Table 19).

Baseline skin to muscle thickness were similar whether administered by pre-filled syringe or auto-injector (mean values of approximately 7.97 mm and 8.81 mm for the PFS and auto-injector groups, respectively, and median of approximately 7.2 mm for both groups). The between-subject variability for this parameter was high (CV% ranged from approximately 54 to 62%) (Table 20). There was no correlation found between mean thigh circumference measurements and the mean skin to muscle thickness measurements regardless of delivery system. There was also no correlation found between mean skin to muscle thickness and mean body mass index measurements.

The loss rate of iohexol from the extravascular tissue compartment ($Loss_{tiss}$) was substantially greater (more than 2X) with the auto-injector delivery system as compared to the PFS (auto-injector mean = 0.585, SD = 0.271; pfs mean = 0.214, SD = 0.252). This suggests a more rapid elimination of iohexol from the tissue from the auto-injector as compared to the PFS which could help explain the early exposure difference in the systemic circulation previously described. The radiodensity of iohexol was similar between the two treatments at the initial 30 second scan (PFS mean = 2744.32, SD = 346.39; auto-injector mean = 2566.18, SD = 573.19);

however, by the 20 minute (600s) scanning time point, there was a large difference between the groups (PFS mean = 2519.44, SD = 615.44; auto-injector mean = 1882.69, SD = 617.87). Radiodensity decreased for both treatment groups over time suggesting elimination of the iohexol from the extravascular tissue compartment into the systemic or possibly lymphatic circulation. In addition, the rate of iohexol loss from the extravascular tissue ($Loss_{Tiss}$) was much greater for the auto-injector as compared to the PFS (auto-injector mean (HU/min) = 0.585, SD = 0.271; pfs mean (HU/min) = 0.214, SD = 0.252) (Table 20).

Table 19: Summary of CT Scanning Dispersion Parameters by Treatment Group

Treatment Group	Statistic	MDd			MWd			DMWd		
		30s (mm)	600s (mm)	1200s (mm)	(30s) (mm)	600s (mm)	1200s (mm)	30s (mm)	600s (mm)	1200s (mm)
Pre-filled Syringe	**n**	10	10	10	10	10	10	10	10	10
	Mean	10.29	9.60	9.59	28.78	32.44	34.48	9.30	8.81	9.01
	SD	4.21	3.79	3.61	6.24	5.94	5.63	3.80	3.11	3.31
	Median	10.35	9.90	10.45	26.55	31.00	33.20	10.15	9.70	9.40
	Min	4.90	4.40	4.20	21.40	25.60	27.80	4.50	4.60	4.20
	Max	17.30	14.40	14.30	42.00	43.90	44.30	16.90	13.20	13.40
	CV (%)	40.88	39.51	37.68	21.69	18.32	16.33	40.90	35.26	36.72
Auto-injector	**n**	10	10	10	10	10	10	10	10	10
	Mean	10.84	10.50	11.60	29.22	32.68	37.01	9.44	9.28	9.63
	SD	3.83	4.53	4.74	8.39	7.94	7.58	3.81	3.81	3.30
	Median	9.90	9.65	10.70	27.85	31.60	34.40	9.25	8.75	9.70
	Min	7.20	5.50	6.70	19.80	22.60	27.10	5.00	5.30	5.30
	Max	18.20	19.30	21.20	50.30	50.80	52.90	16.00	17.50	16.60
	CV (%)	35.32	43.14	40.90	28.70	24.28	20.48	40.36	41.05	34.29

MDd = Maximum Depth of Dispersion, MWd = Maximum Width of Dispersion, DMWd = Depth at Maximum Width of Dispersion

Table 20: Summary of CT Scanning Radiodensity Parameters by Treatment Group

Treatment Group	Statistic	Baseline Skin to Muscle Thickness (mm)	$Loss_{Tiss}$ (HU/min)	Radiodensity		
				30s (HU)	600s (HU)	1200s (HU)
Pre-filled Syringe	**n**	10	10	10	10	10
	Mean	7.97	0.214	2744.32	2656.51	2519.44
	SD	4.33	0.252	346.39	566.63	615.44
	Median	7.15	0.113	2883.58	2868.32	2818.59
	Min	2.60	0.015	2070.12	1285.62	1081.05
	Max	16.10	0.841	3067.95	3047.39	3050.05
	CV (%)	54.35	117.52	12.62	21.33	24.43
Auto-injector	**n**	10	10	10	10	10
	Mean	8.81	0.585	2566.18	2293.68	1882.69
	SD	5.49	0.271	573.19	635.57	617.87
	Median	7.20	0.588	2853.63	2406.37	1929.57
	Min	3.20	0.048	1651.05	1120.49	1038.96
	Max	18.90	1.06	3051.63	3028.98	2990.25
	CV (%)	62.26	46.27	22.34	27.71	32.82

$Loss_{Tiss}$ = Iohexol loss rate from extravascular tissue

Mixed model assessment of computed tomography parameters. Mixed-effects models were used to test for differences in the mean changes over time (30s, 600s, 1200s) between the delivery systems as well as differences between delivery systems at each individual time point with respect to the maximum depth at dispersion (MDd), maximum width of dispersion (MWd), depth at maximum width of dispersion (DMWd), and radiodensity after controlling for period and sequence effects as well as age, sex,, thigh circumference, and baseline skin to muscle thickness. The models included a random subject effect to account for within subject variations. Furthermore, the models included fixed effects for treatment, time, treatment by time, period, and sequence as well as the aforementioned additional covariates. Each of these covariates were

included in the model in order to determine what effects each, or any may have on the response parameters (MDd, MWd, DMWd, and radiodensity) by treatment group. Using these models, the mean response was estimated at each time point by treatment group and the mean changes over time were compared statistically between the groups using the treatment by time interaction effect at a significance level of $\alpha = 0.05$ (Tables 21 and 22).

Table 21: (Adjusted) Mean Dispersion Response Measures by Treatment Group

		Auto-injector			Pre-filled Syringe		
Response	**Time (s)**	**Mean**	**SE**	**95% CI**	**Mean**	**SE**	**95% CI**
MDd (mm)	**30**	10.69	1.00	(8.17, 13.20)	11.35	1.01	(8.72, 13.78)
	600	10.35	1.00	(7.83, 12.86)	10.56	1.01	(8.03, 13.09)
	1200	11.45	1.00	(8.93, 13.96)	10.55	1.01	(8.02, 13.08)
MWd (mm)	**30**	28.96	2.35	(23.98, 33.95)	28.11	2.38	(23.03, 33.18)
	600	32.42	2.35	(27.44, 37.41)	31.77	2.38	(26.69, 36.84)
	1200	36.75	2.35	(31.77, 41.74)	33.81	2.38	(28.73, 39.88)
DMWd (mm)	**30**	9.20	0.69	(7.65, 10.76)	9.85	0.70	(8.27, 11.42)
	600	9.04	0.69	(7.49, 10.60)	9.36	0.70	(7.78, 10.93)
	1200	9.39	0.69	(7.84, 10.95)	9.56	0.70	(7.98, 11.13)

CT maximum depth of dispersion (MDd). There was not a significant treatment by time interaction effect on the maximum dispersion depth ($p = 0.3793$); that is, the changes in MDd over time did not differ significantly between the two treatment groups. Additionally, there were no significant treatment or time effects (that is, the PFS and auto-injector groups were not different, irrespective of time, and there were no changes over time irrespective of the delivery system utilized). The adjusted mean MDd is summarized and plotted over time by each treatment group in Table 21 and **Figure 49**, respectively. There was a significant effect of skin to muscle thickness on MDd ($p < 0.0001$). For every one mm increase in subject skin to muscle

thickness the maximum depth of dispersion increased by 1.32 mm (SE = 0.23, 95% CI = 0.84, 1.80). Therefore, the greater the distance between skin and muscle (or the larger the subcutaneous tissue layer), the greater the depth of dispersion regardless of treatment group. No other covariates were found to have an effect on MDd.

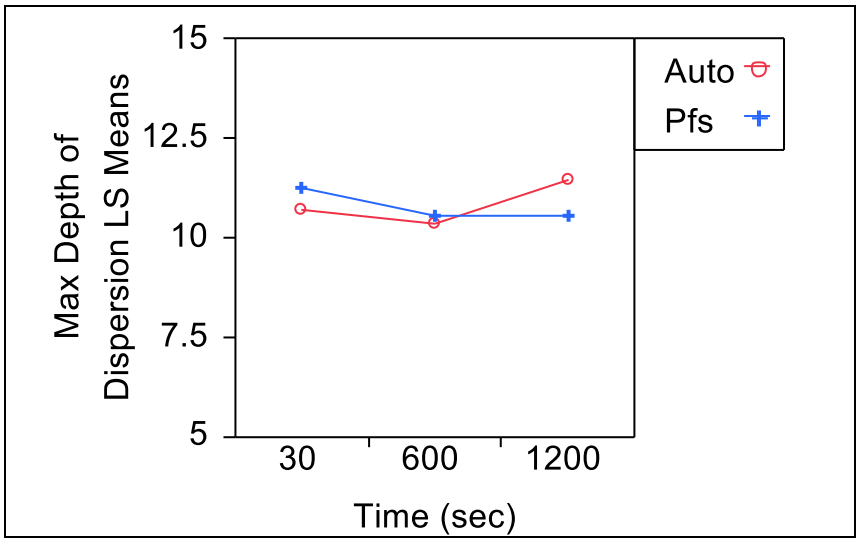


Figure 49: Adjusted Changes in MDd (mm) over Time (seconds) by Treatment Group

CT maximum width of dispersion (MWd). There was not a significant treatment by time interaction effect on the maximum width of dispersion (MWd) ($p = 0.8028$); that is, the changes in MWd over time did not differ significantly between the two treatment groups. There was not a significant treatment effect on MWd (that is, iohexol delivery by PFS or auto-injector were no different, irrespective of time); however, there was a significant time effect on MWd (that is, there were increases in MWd over time, irrespective of delivery system) ($p = 0.0042$). The adjusted mean MWd is summarized and plotted over time by each treatment group in Table 21 and **Figure 50**, respectively. There were no significant effects of any of the other covariates on MWd.

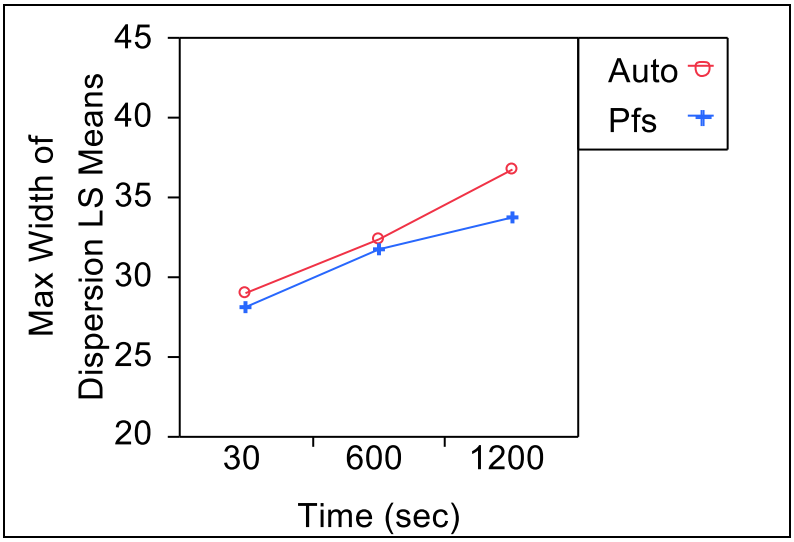


Figure 50: Adjusted Changes in MWd (mm) over Time (seconds) by Treatment Group

CT depth at maximum width of dispersion (DMWd). There was not a significant treatment by time interaction effect on the depth at maximum width of dispersion (DMWd) (p = 0.8731); that is, the changes in DMWd over time did not differ significantly between the two treatment groups. Mean DMWd is summarized and plotted over time by each treatment group in Table 21 and **Figure 51**, respectively. There were no significant treatment or time effects (that is, the delivery system groups were not different, irrespective of time, and there were not changes over time, irrespective of delivery system). There was a significant effect of skin to muscle thickness on DMWd ($p < 0.0001$). For every one mm increase in subject skin to muscle thickness the depth at maximum width of dispersion increased by 0.93 mm (SE = 0.18, 95% CI = 0.55, 1.31). Therefore, the greater the distance between skin and muscle (or the larger the subcutaneous tissue layer), the greater the depth at maximum width of dispersion irrespective of treatment group.

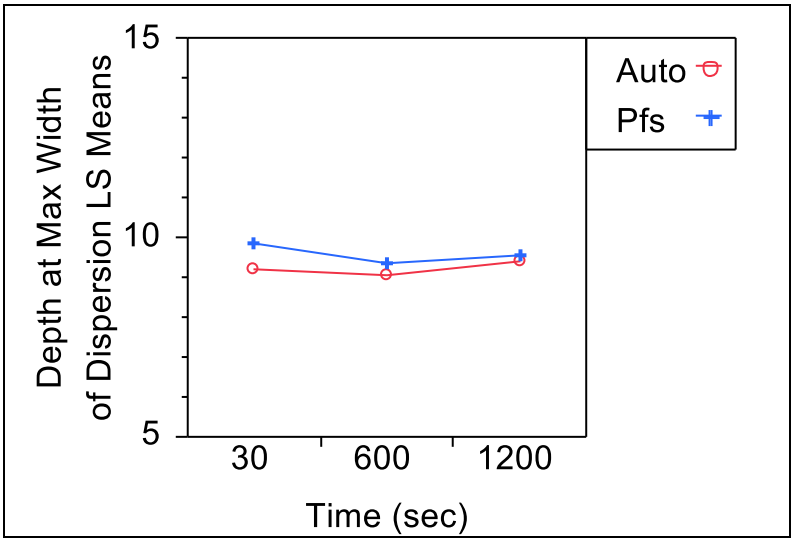


Figure 51: Adjusted Changes in DMWd (mm) over Time (seconds) by Treatment Group

Radiodensity. Mean iohexol radiodensity is summarized and plotted over time by each delivery system treatment group in Table 22 and **Figure 52**, respectively. There was not a significant treatment by time interaction effect ($p = 0.2734$); that is, the changes in radiodensity over time did not differ significantly between the two treatment groups. There was a significant treatment/delivery system effect on the elimination of iohexol as measured by radiodensity ($p = 0.0002$); that is, there were significant differences between the delivery system treatment groups, regardless of time. Specifically, the Auto-injector treatment group had a significantly lower iohexol radiodensity measurement as compared to the PFS treatment group at both the 10 minute (600 s) and 20 minute (1200 s) scanning time points (Radiodensity difference at 600s = 469.55, 95% CI = 55.72, 883.38; Radiodensity difference at 1200s = 743.46, 95% CI = 329.63, 1157.29) (Table 23). There was a significant time effect ($p = 0.0095$). That is, there were decreases in radiofrequency over time, irrespective of treatment group. No other covariates had an effect on radiodensity.

Table 22: (Adjusted) Mean Radiodensity Response Measures by Treatment Group

Treatment Group	Time (s)	Mean	SE	95% CI
Auto-injector	**30**	2565.33	190.78	(2110.30, 3020.37)
	600	2292.83	190.78	(1837.80, 2747.86)
	1200	1881.85	190.78	(1426.81, 2336.88)
Pre-filled Syringe	**30**	2850.20	194.58	(2386.59, 3313.80)
	600	2762.38	194.58	(2298.77, 3225.98)
	1200	2625.31	194.58	(2161.70, 3088.92)

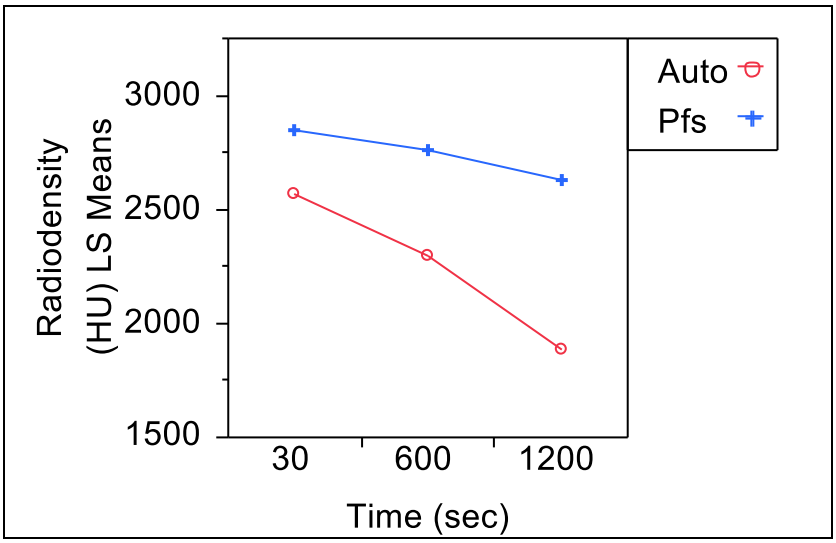


Figure 52: Adjusted Changes in Radiodensity (HU) over Time (seconds) by Treatment Group

Table 23: (Adjusted) Mean Differences (PFS – Auto-injector) in Radiodensity Measures

	Radiodensity (HU)				
Time	**Difference**	**SE**	**95% CI**	**p-value**	
30 s	284.86	204.87	(-128.97, 698.69)	0.1719	
600 s	469.55	204.87	(55.72, 883.38)	0.0272	†
1200 s	743.46	204.87	(329.63, 1157.29)	0.0008	†

† Indicates statistically significant at $\alpha = 0.05$

Mixed model assessment of Iohexol loss rate from the extravascular tissue. A Mixed-effect model was also used to test for differences in the iohexol loss rate from the extravascular

tissue compartment ($Loss_{Tiss}$) between the Auto-Injector and PFS delivery systems after controlling for the covariates of period, sequence effects, age, sex, and thigh circumference. The models included a random subject effect to account for within subject variations and fixed effects for treatment, period, and sequence as well as the aforementioned covariates. Each of these covariates were included in the model in order to determine what effects each, or any may have on the loss rate by delivery system treatment group. Using this model, the mean responses were estimated for each group and the differences in the means were compared statistically between the groups using a significance level of $\alpha = 0.05$.

The mixed-effects model accounted for 71.3% variations in the iohexol extravascular tissue loss rate ($Loss_{Tiss}$)(r^2 adjusted = 50.4%). There was a significant treatment effect on the loss rate of iohexol from the extravascular tissue compartment. Mean $Loss_{Tiss}$ for the Auto-injector treatment group was significantly higher than the PFS treatment group (auto-injector mean $Loss_{Tiss}$ = 0.562, 95% CI = 0.315, 0.809; PFS mean $Loss_{Tiss}$ = 0.191, 95% CI = -0.056, 0.438). The mean difference between treatments was 0.37 HU/min (95% CI = 0.14, 0.60; p = 0.0058) confirming the elimination of iohexol from the subcutaneous or intramuscular tissue occurred significantly faster with the auto-injector as compared to the PFS. None of the other covariate, including the physiologic/non device-related covariates of age, sex and thigh circumference has an effect on the iohexol extravascular tissue loss rate.

Comparison of Tissue Bioavailability and Dispersion Characteristics with the Systemic Exposure of Iohexol

As discussed in the Analysis of Early Exposure section, there was greater early exposure of iohexol into plasma (i.e., systemic circulation) with the auto-injector as compared with the pre-filled syringe (See Mixed Effect Model $AUC_{0\text{-}40}$ and $AUC_{0\text{-}60}$ analysis within this earlier section). Because the change in radiodensity over time at the injection site can be thought of as a measure for the loss rate of iohexol from the extravascular compartment into the systemic circulation ($Loss_{Tiss}$), one would have anticipated the auto-injector to have a significantly lower radiodensity level as compared to the PFS at time points prior to 60 minutes, as was indeed the case for the 600s (10 min) and 1200s (20 min) time points as shown in Table 23.

As a result of these findings, a local *in-vivo*$_{(\text{extravascular disappearance})}$, systemic *in-vivo*$_{(\text{intravascular appearance})}$ correlation ($IV_{(extra)}IV_{(intra)}C$) was conducted to assess the rate of iohexol loss from the extravascular tissue compartment ($Loss_{Tiss}$) relative to the extent of early plasma exposure ($AUC_{0\text{-}30,}$ $AUC_{0\text{-}40}$ and $AUC_{0\text{-}60}$ and $AUC_{0\text{-}Tmax}$) between the two delivery systems. There was a significant positive correlation between the loss rate of iohexol from the extravascular tissue compartment (using radiodensity as a measure for the loss of iohexol from this compartment) and the extent of early plasma exposures up to 60 minutes ($AUC_{0\text{-}30,}$ $AUC_{0\text{-}40}$ and $AUC_{0\text{-}60}$) with the auto-injector treatment group. Therefore, higher rates of iohexol extravascular elimination, presumably reflecting uptake into the intravascular compartment, were correlated with a higher extent of early iohexol plasma exposures when administered by the auto-injector delivery system. This significant correlation was not present when comparing the loss

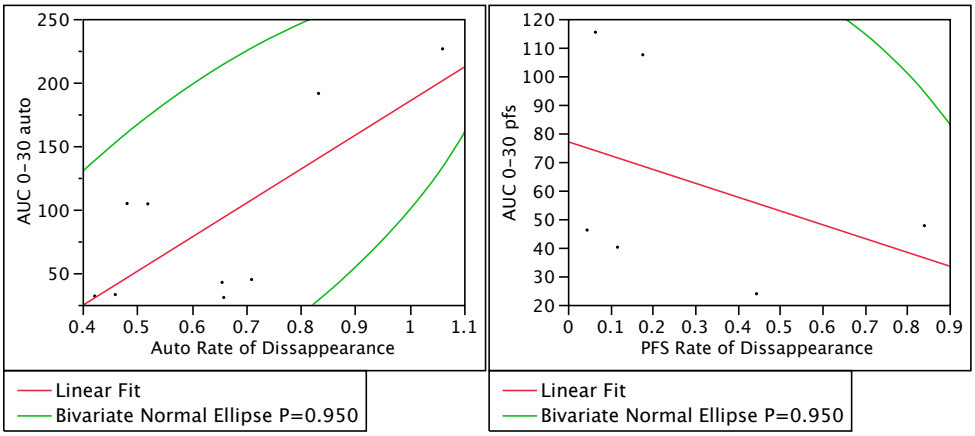
rate from the extravascular tissue with $AUC_{0\text{-}Tmax}$ possibly due to iohexol plasma levels equilibrating by the time C_{max} was reached. Additionally, there was no correlation found between the loss rate of iohexol from the extravascular tissue compartment and the extent of early iohexol plasma exposures at any of these early AUC metrics for the PFS treatment group. This confirms that the delivery system had an effect on the disappearance of iohexol from the injection site when compared to the appearance of iohexol in the vasculature as measured by the extent of early plasma exposure. A summary of the $IV_{(extra)}IV_{(intra)}C$ results and associated levels of significance may be found in Table 24.

Table 24: Summary of $IV_{(extra)}IV_{(intra)}C$ Parameters

	Auto-Injector				Pre-filled Syringe			
	$Loss_{Tiss}$ by $AUC_{0\text{-}30}$	**$Loss_{Tiss}$ by $AUC_{0\text{-}40}$**	**$Loss_{Tiss}$ by $AUC_{0\text{-}60}$**	**$Loss_{Tiss}$ by $AUC_{0\text{-}Tmax}$**	**$Loss_{Tiss}$ by $AUC_{0\text{-}30}$**	**$Loss_{Tiss}$ by $AUC_{0\text{-}40}$**	**$Loss_{Tiss}$ by $AUC_{0\text{-}60}$**	**$Loss_{Tiss}$ by $AUC_{0\text{-}Tmax}$**
r^2 (%)	74.6	79.0	83.7	5.8	-3.9	1.0	14.6	-24.6
Slope	276.92	454.68	961.11	798.41	-48.36	2.50	82.74	-598.19
***p*-value**	0.021	0.011	0.005	0.078	0.441	0.982	0.707	0.494

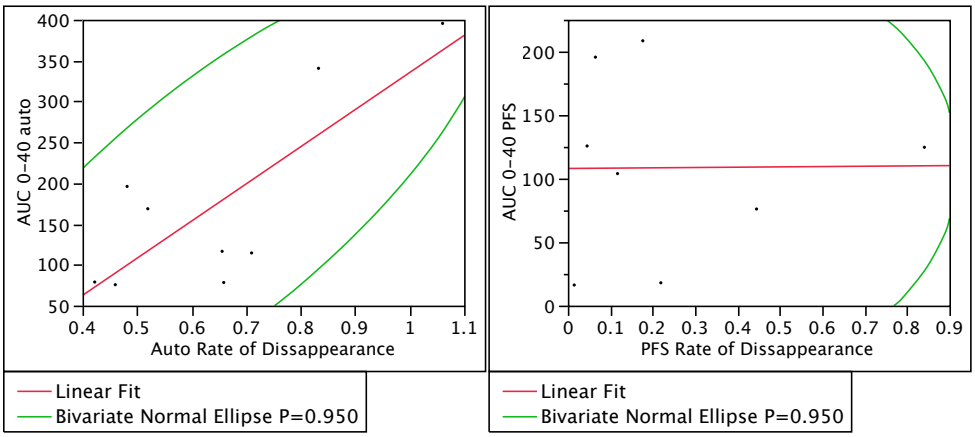
Units for AUC = ug.min/mL, Units for $Loss_{Tiss}$/Rate of Disappearance = HU/min

Representative linear regression plots for the Auto-injector as compared to the Pre-filled syringe may be found in Figures 53 and 54 below.



AUC (ug.min/mL), $Loss_{Tiss}$/Rate of Disappearance (HU/min)

Figure 53: Bivariate Fit of AUC_{0-30} by $Loss_{Tiss}$ (Left: Auto-injector, Right: PFS)



AUC (ug.min/mL), $Loss_{Tiss}$/Rate of Disappearance (HU/min)

Figure 54: Bivariate Fit of AUC0-40 by LossTiss (Left: Auto-injector, Right: PFS)

Because the extent of early exposure of iohexol was found to be different between the auto-injector and PFS delivery systems at the early exposure time points, especially AUC_{0-40} and $AUC_{0-60,}$ and because the radiodensity measurements were also found to be different between the delivery systems, these differences were evaluated further for a relationship. The radiodensity parameter was chosen at 20 minutes to compare with each partial AUC measurement because the

AUC time point range was beyond this last, 20 minute, CT scanning time point. Therefore, the difference in radiodensity at 20 minutes was compared with the differences between the early exposure parameters, $AUC_{0\text{-}30,}$ $AUC_{0\text{-}40}$ and $AUC_{0\text{-}60}$ and $AUC_{0\text{-}Tmax}$.

There was a significant positive correlation between the difference in radiodensity at 20 min and the difference in $AUC_{0\text{-}30}$ values between the delivery systems; that is larger differences in radiodensity between the auto-injector and PFS at 20 minutes were associated with larger differences in $AUC_{0\text{-}30}$ between the delivery systems (r^2 = 86.5%, p = 0.0261). This was also the case with $AUC_{0\text{-}60}$ and $AUC_{0\text{-}Tmax}$ ($AUC_{0\text{-}60}$ r^2 = 73.2%, p = 0.0389; $AUC_{0\text{-}Tmax}$ r^2 = 73.2%, p = 0.0389) and was borderline significant for $AUC_{0\text{-}40}$ (r^2 = 75.3%, p = 0.0507).

In summary, the differences in radiodensity at the 20 minute time point between the PFS and auto-injector had a positive correlation with most all of the early exposure measurement differences between the delivery systems. Iohexol delivery by the auto-injector had a significant, positive $IV_{(extra)}IV_{(intra)}C$ which was not present when iohexol was administered by the pre-filled syringe. This difference may be due to the extravascular loss rate itself which was significantly higher for the auto-injector as compared to the PFS. Because the only difference between the treatment groups in this study was the nature of the delivery system, providing either a manual injection (PFS) or automated injection (auto-injector) of the iohexol, these findings suggest device-related factors such as the kinematics of injection (including associated fluid dynamics and injection force) may have had an effect on the resultant systemic exposure behavior of the drug.

Safety Evaluation

The safety population was defined as all subjects who received at least one dose of iohexol. Ten subjects were included in the safety population. A summary of TAEs is presented in Table 25. There were no deaths or TEAEs. Iohexol delivery by either the pre-filled syringe or auto-injector was well tolerated by all subjects with only mild, injection site-related adverse events reported. Subjects experienced more mild adverse events with the auto-injector (8 events in 6 subjects) as compared to the pre-filled syringe (3 events in 3 subjects). A summary of events by treatment group and event classification/description may be found in Table 26. All events resolved spontaneously prior to study discharge.

Table 25: Summary of TEAEs by Delivery System

	Treatment Group	
	Pre-filled Syringe N = 10 n (%) e	**Auto-injector N = 10 n (%) e**
All TEAEs	3 (30.0) 3	6 (60.0) 8
'Related' TEAEs	3 (30.0) 3	6 (60.0) 8
Mild TEAEs	3 (30.0) 3	6 (60.0) 8
Moderate TEAEs	0	0
Severe TEAEs	0	0
Deaths	0	0
Serious TEAEs	0	0
TEAEs leading to Withdrawal	0	0

N = number of subjects; n = number of subjects with treatment emergent adverse event; e = number of separate events; % = percentage of subjects experiencing event (n/N X 100); TEAE = treatment emergent adverse event; "related" = definite, probable, possible

Table 26: Number of Subjects Experiencing Treatment Emergent Adverse Events and Percentage of Subjects per Exposure (i.e. injection) by Treatment Group and Event Description

Event Description	Treatment Group	
	Pre-filled Syringe n (%)	Auto-injector n (%)
General Injection Site Reactions	3 (30.0)	8 (80.0)
Injection Site Discomfort	0 (0.0)	1 (10.0)
Injection Site Swelling	0 (0.0)	1 (10.0)
Injection Site Bleeding	0 (0.0)	2 (20.0)
Injection Site Induration	1 (10.0)	1 (10.0)
Injection Site Pain	2 (20.0)	1 (10.0)
Injection Site Paraesthesia	0 (0.0)	1 (10.0)
Injection Site Burning	0 (0.0)	1 (10.0)

n = number of subjects with treatment emergent adverse event; % = percentage of subjects experiencing event (n/N X 100)

Conclusion

The primary objective of this randomized, single-dose, crossover study was to assess the ability of a radioimaging approach combined with an analysis of systemic exposure measurements to serve as a novel approach for investigating sources of variability that may be introduced by injectable delivery systems. This was accomplished by comparing qualitative and quantitative *in-vivo* injection dispersion parameters from computed tomography (CT) scanning with systemic exposure metrics following administration of a non-ionic radiocontrast media, iohexol (Omnipaque®). The discriminatory capacity of the approach was assessed by comparing iohexol administered subcutaneously by an auto-injector delivery system versus a pre-filled syringe delivery system.

Twelve subjects were randomized to one of two possible treatment sequences (Auto-injector first, pre-filled syringe second or pre-filled syringe first, auto-injector second) to determine the order in which they would receive the iohexol in Periods 1 or 2. The first two subjects were included in an interim analysis set to evaluate the usability of the iohexol assay as well as the Computed Tomography images. The final analysis was conducted with the remaining ten subjects, after adjusting the iohexol dose, plasma sampling schedule and CT scanning schedule. None of the subjects had pre-existing conditions that prohibited them from participation and all met the inclusion/exclusion criteria.

Serial blood sampling was performed relative to dosing in each study period, and the systemic exposure parameters were calculated from each subject's plasma iohexol concentration-time profiles. Computed tomography scanning was also performed relative to dosing and the CT qualitative and quantitative parameters were assessed through non-compartmental analysis from each subject's radioimaging series. Iohexol peak (C_{max}) and total (AUC_{0-t}, $AUC_{0-\infty}$) exposure were similar whether administered by pre-filled syringe or auto-injector. However, partial area data (AUC_{0-40}, AUC_{0-60}) relative to the auto-injector and pre-filled syringe were significantly different, suggesting early exposure differences between the two delivery systems. Additionally, although the dispersion measurements obtained were also similar whether administered by pre-filled syringe or auto-injector, changes in radiodensity over time, as a measure of the loss of iohexol from the extravascular tissue compartment into the systemic circulation, were significantly different between the two delivery systems.

Finally, a local *in-vivo*$_{(\text{extravascular disappearance})}$, systemic *in-vivo*$_{(\text{intravascular appearance})}$ correlation ($IV_{(extra)}IV_{(intra)}C$) was conducted to assess the rate of iohexol loss from the extravascular tissue compartment ($Loss_{Tiss}$) relative to the extent of early plasma exposure ($AUC_{0\text{-}30,}$ $AUC_{0\text{-}40}$ and $AUC_{0\text{-}60}$ and $AUC_{0\text{-}Tmax}$) between the two delivery systems. There was a significant positive correlation between the loss rate of iohexol from the extravascular compartment and the extent of early plasma exposure with the auto-injector treatment group. This was not present with the pre-filled syringe treatment group. Therefore, higher rates of iohexol extravascular elimination, presumably reflecting uptake into the intravascular compartment, were correlated with a higher extent of early iohexol plasma exposures when administered by the auto-injector delivery system.

These data show that changes in the manner that an injectable is administered may have an impact on the systemic exposure of a drug as a result of changes in the behavior of the drug in the tissue. Iohexol administration by either delivery system was well tolerated with minimal adverse events being solely related to injection site reactions that resolved spontaneously without intervention. This study provided early data that CT scanning with an analysis of systemic exposure metrics using iohexol as an injectable standard may be used to assess sources of variability from different injectable delivery systems.

CHAPTER 5. DISCUSSION AND FUTURE RESEARCH

Discussion

This dissertation seeks to provide an initial, novel approach for understanding what occurs at the moment of injectable drug delivery deposition into the tissue along with an assessment of systemic exposure, as well as the relationship between these two events. The radioimaging approach utilized in this study, computed tomography scanning, coupled with a non-ionic contrast media, provided a unique method for the evaluation of tissue dispersion characteristics. This included an assessment of the injectate location and the ability to quantify depth and width of penetration. Furthermore, this approach allowed for the detection of changes in these parameters over time. This is important when trying to correlate tissue compartment elimination of an injectable with the absorption of the injectate into the systemic circulation.

Pharmaceutical scientists have focused decades of research and development on the manipulation of an injectable formulation itself to impact the systemic exposure and resultant pharmacodynamic behavior of a drug; however, there has been very little focus on modifying delivery mechanics in order to adjust the desired behavior of a drug. Traditionally, devices for injectable delivery have been viewed as "secondary packaging" components for an injectable drug/device combination product. The primary goal was to ensure an injectable reached its target site. For example, needle-free injectors must demonstrate that the fluid jet force through a micro-orifice is sufficient to pierce the skin and deposit an injectate beneath. However, little has been

done to understand how changes in the kinematics of injection, as driven by changes in device-related factors, affect injectable formulation characteristics and the resultant drug behavior. This is in stark contrast to inhaled drug product development, where scientists have understood the need to focus not only on formulation factors, but also on variables related to device engineering that can affect drug deposition or dispersion and the resultant systemic compartment behavior *in-vivo* for decades. This dissertation research provides a paradigm that may be leveraged to begin evaluation of these device-related dynamics in injectable product development.

There were several notable findings of this research. First and foremost, if the study analysis only included an assessment of the conventional exposure parameters, C_{max}, T_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, the results would have indicated a similar profile between the auto-injector and pre-filled syringe. By incorporating the CT measurement of radiodensity as a measure of iohexol loss from the extravascular tissue into the systemic circulation, it was clear that there was a difference in drug behavior present between the delivery systems. This was confirmed by evaluating early exposure parameters closer to the CT scanning time points where these radiodensity changes were apparent (CT measurements only occurred up to 20 minutes post injection). This evaluation demonstrated a difference in early systemic exposure. The differences were likely device-related because the covariates of age, sex and thigh circumference analyzed had no effect on the rate of loss of iohexol from the extravascular tissue into the system circulation or on the early iohexol plasma exposure parameters ($AUC_{partial}$). This is an important finding as differences in early systemic exposure may be clinically relevant for certain classes of

drugs. Examples include acute-care injectables for life-threatening emergencies such as epinephrine for anaphylaxis, glucagon for severe hypoglycemia, and certain benzodiazepines for acute repetitive seizures where a greater extent of early exposure may result in therapeutically beneficial pharmacodynamic changes independent of the time to maximum plasma concentration (T_{max}). Alternatively, there are some pharmaceuticals whereby a greater early extent of exposure may be harmful to a patient. In this study, iohexol was shown to have a higher early systemic exposure with an auto-injector as compared to a PFS method of administration. If the iohexol results were translatable to other drugs with similar physicochemical properties indicated for the treatment of a life-threatening emergency, the earlier exposure achieved by the auto-injector may have been clinically meaningful.

It is important to note the radiographic and systemic exposure differences seen with iohexol in this study between the auto-injector and the pre-filled syringe are not necessarily translatable to other drugs. For example, another contrast media agent with different physicochemical properties may behave differently if administered by the same delivery systems evaluated in this study. Alternatively, iohexol may behave differently if new variables were assessed such as having different individuals administer the injections versus the same individual, or even having iohexol being injected using other delivery systems. This is one advantage of the approach as it allows for the assessment of tissue dispersion and systemic exposure metrics across any number of delivery techniques or systems.

Another notable finding was the difference in dispersion patterns obtained from the interim subject population as compared to the post-interim subject population. Specifically, the dose of 150 mg delivered in 0.5 mL by the auto-injector during the interim period as compared to the 300 mg dose delivered in 1.0 mL by the auto-injector in the post-interim period resulted in a noticeably deeper iohexol penetration (Figure 55).

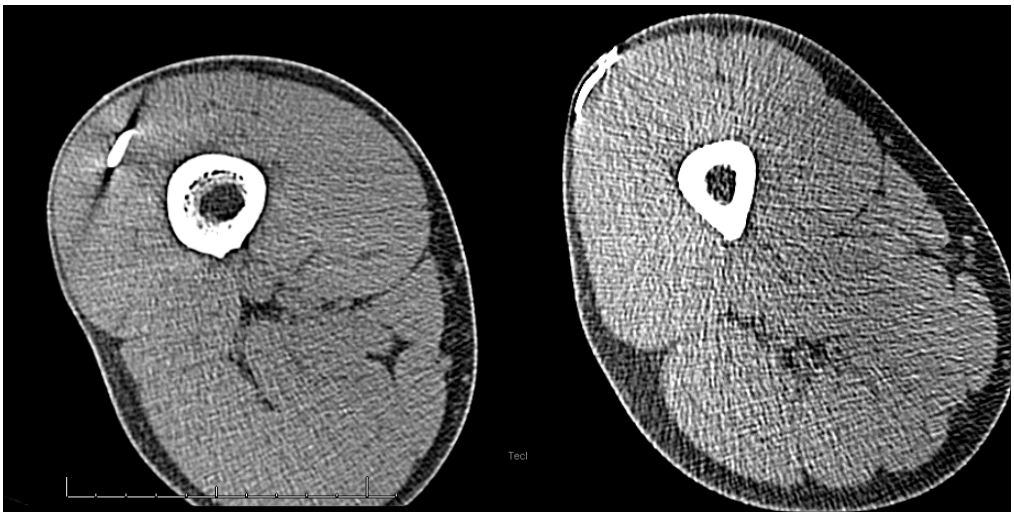


Figure 55: Dispersion by Auto-injector Delivery System at 10 minutes following a 150mg/0.5mL Iohexol Dose (Left) as compared to a 300 mg/1.0mL Iohexol Dose (Right)

Upon further evaluation of the mechanics surrounding the Autoject 2® delivery system, there was a substantial difference discovered in the distance between the loaded spring and the piston, when setting a volume of 0.5 mL for delivery as compared to 1.0 mL. The spring load in the auto-injector has a certain amount of potential energy that is available to act on the syringe resulting in the delivery of the injectate. This potential energy is the same regardless of the

position of the piston; however, due to the gap created from setting the piston at a lower level in the syringe to allow for a 0.5 mL volume as compared to a 1.0 mL volume, there may be more kinetic energy applied during actuation to the 0.5 mL dose. This would result in a greater force against the piston and subsequent higher fluid jet force exiting the needle orifice when against the injection site. This may explain the substantial difference in the deposition of the iohexol in the muscle with the first two subjects (interim population) as compared to the remaining ten subjects (post-interim population) where the vast majority of injections were located subcutaneously. These mechanical and kinematic, device-related factors, warrant further investigation as a result of this finding as there are substantial clinical implications if there is a difference in dispersion characteristics that may be solely attributable to the variable of volume administered.

The local *in-vivo*$_{(appearance)}$, systemic *in-vivo*$_{(disappearance)}$ correlation was only applicable to the auto-injector as compared to the pre-filled syringe. Because the rate of iohexol tissue loss was also significantly greater for the auto-injector as compared to the pre-filled syringe, it is presumed that this is the determining factor for achieving the correlation. As such, one would expect that the incorporation of another pharmaceutical formulation which had a similar $Loss_{Tiss}$ profile using the same auto-injector, would also result in a positive correlation.

This is the first known study describing the systemic exposure of iohexol delivered subcutaneously. The pharmacokinetics of iohexol have been previously studied through other routes of administration including the intravenous and intrathecal routes. The iohexol product

monograph provides a terminal elimination half-life of approximately 12 hours when administered intravenously and 4.5 hours following a subarachnoid injection (Omnipaque (Iohexol) Prescribing Information. General Electric Company. 2008). This is in contrast to other pharmacokinetic studies of iohexol. For example, a study by Olsson and colleagues that injected multiple doses of iohexol intravenously resulted in a terminal elimination half-life of between 2 and 6 hours and peak plasma concentrations of 10 to 50 µg/mL (Olsson *et al.,* 1983). These are similar to the values reported in this research study assessing these exposure parameters with mean plasma $T_{1/2}$ occurring at approximately 2 hours and peak plasma concentrations of 2 -30 µg/mL (mean of approximately 17 ug/mL).

For years, pharmaceutical research scientists have been developing parenteral dosage forms and have been testing these products in clinical trials with, for the most part, traditional pharmacokinetic analyses. A large reason for this has been the regulatory requirements for the conduct and execution of clinical studies to support marketing applications. As is the case with most pharmaceutical development programs, regardless of the dosage form, the majority of these injectable product clinical trials are conducted in controlled settings, with well-trained administrators providing injections to subjects that ensure compliance with the desired administration regimen and delivery techniques. However, with the relatively recent explosion of injectable products being moved out of the healthcare environment administration setting into the home environment for administration by laypersons or caregivers, the questions must be asked:

- How do we know that the clinical trials completed in the controlled trial setting to support marketing applications are characteristic of what occurs in the out-of-hospital environment?
- How can we ensure that safety and efficacy results are translatable into a setting whereby numerous variables are introduced that weren't controlled for in the clinical trials conducted to support original marketing approval?
- What is the impact of the additional variables, introduced by device and administration-related factors unique to injectable products, on the safety and efficacy of a drug product (being that the risk of noncompliance with an administration regimen are only amplified by the injectable route versus a conventional oral route of administration)?

These and other similar questions can only be answered by having a complete understanding of the mechanics of tissue deposition and dispersion, along with systemic absorption and distribution parameters that may be affected by various administration factors introduced by injectable drugs and their associated means of delivery.

Future Research Directions

This work provides a glimpse into numerous possibilities for investigating sources of pharmacokinetic variability that may be introduced by injectable drugs and associated delivery systems or mechanisms. As this research was an initial pilot study, further work is required to

validate the approach and to assess its usability on a greater scale. One limitation of this work includes the choice of iohexol as the injectable standard. This approach allows a research scientist to evaluate the impact of changing certain device and related variables on the behavior of drugs identical or similar to the physicochemical properties of iohexol; however, this must be proven to be reproducible with drugs other than iohexol in additional studies. One approach would be to formulate iohexol to closely match the physicochemical characteristics of another compound, followed by conducting a cross-over study with both drugs, assessing the dispersion of iohexol and the resultant bioavailability of each, within and between subjects. Another limitation of this study was the use of a CT program that was built for clinical assessment and diagnosis as compared to being optimized for pharmaceutical research. The image processing software requires manual measurements of the dispersion and radiodensity parameters for each individual CT slice, resulting in calculations based on the image processing program tools for the given slice assessed. Optimizing the software to automatically provide the dispersion and radiodensity measurements across the entire series of images for a given subject would reduce potential human error, save time, and improve the robustness of the analysis. In addition, due to the absence of a dedicated CT scanner for research purposes, scanning times were limited for each subject resulting in scanning time points that could not exceed 20 minutes per subject. Further research should be conducted with scanning time points further out and more closely aligned to the plasma sampling time points.

As there was a difference in the rate of loss of iohexol in this study relative to the delivery system chosen for administration, further work needs to be conducted to assess which dispersion parameters likely had an effect on this loss rate. Furthermore, because there appeared to be a difference in dispersion as a result of changes in device-related factors, such as piston placement that determines volume administered, research should be conducted to understand which factors (e.g. piston placement, spring force, needle gauge or length) have the greatest impact on dispersion and the associated systemic exposure parameters following administration.

Similarly, this approach allows scientists to investigate the impact of formulation factors (e.g. *pH*, osmolality, viscosity, molecular weight, partition coefficient, inclusion of an extended release vehicle) on dispersion parameters. This study attempted to manipulate one variable, the choice of delivery system. It is clear that this approach could be used to investigate other administration-related variables such as differences between injection techniques by trained nurses, or the differences between healthcare practitioner and layperson administration using an identical delivery system.

In conclusion, this research resulted in a novel local *in-vivo*$_{(\text{extravascular disappearance})}$, systemic *in-vivo*$_{(\text{intravascular appearance})}$ correlation approach that could assess a wide variety of dynamics associated with injectable drug delivery below the dermis. The work demonstrated the feasibility of combining radioimaging approaches with traditional systemic exposure metrics to achieve a greater understanding of the *in-vivo* behavior of injectable drugs and their associated delivery systems.

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APPENDIX A-1

Final IRB Approval

VCU Memo

Virginia Commonwealth University

Office of Research Subjects Protection
Bio-Tech Research Park, Building 1
800 E. Leigh St., Ste.#114
P.O. Box 980568
Richmond, Virginia 23298-0568

DATE: May 9, 2011

TO: William Garnett, PharmD
Pharmacotherapy and Outcome Sciences
Box 980533

FROM: Andrea Hastillo, MD
Chairperson, VCU IRB Panel C
Box 980568

RE: **VCU IRB #: HM13424**
Title: Development of a Novel Model to Assess Qualitative and Quantitative Injectable Delivery Dynamics

On May 5, 2011 the minor changes to your research study were ***approved*** in accordance with 110 (b) (2). This approval includes the following items reviewed by this Panel:

PROTOCOL: Development of a Novel Model to Assess Qualitative and Quantitative Injectable Delivery Dynamics (Version #11EPHD.007 dated 5/4/11, stamped received 5/5/11)

As a reminder, the approval for this study expires on March 2, 2012. Federal Regulations/VCU Policy and Procedures require continuing review prior to continuation of approval past that date. Continuing Review report forms will be mailed to you prior to the scheduled review.

The Primary Reviewer assigned to your research study is V. Ramana Feeser, MD. If you have any questions, please contact Dr. Feeser at vrfeeser@aol.com or 828-5250; or you may contact Clara Cline, IRB Coordinator, VCU Office of Research Subjects Protection, at ccline3@vcu.edu or 827-1446.

APPENDIX A.2

Radiation Safety Section Approval

VCU Memo

Virginia Commonwealth University

MCV Campus

Office of Environmental Health and Safety
Radiation Safety Section

Sanger Hall
1101 E. Marshall Street
P.O. Box 980112
Richmond, Virginia 23298-0112

804 828-9131
Fax: 804 828-1157

Date: November 12, 2010

To: John N. Clore, M.D.
Internal Medicine

William R. Garnett, Pharm.D.
Department of Pharmacy

From: Dean W. Broga, Ph.D., Director
Radiation Safety Officer

Subject: **Application #10-26H - "Development of a Novel Model to Assess Qualitative and Quantitative Injectable Delivery Dynamics"**

Your application has been reviewed and approved by the Radiation Safety Officer. At this time, you may proceed with your study, pending VCU Office of Research Subjects Protection approval. For information purposes, the entire committee will discuss your application at their next meeting on December 9, 2010. **Please note that this approval applies only to studies performed at VCU/VCUHS.**

If you have any questions or comments, please do not hesitate to call me.

Appendix B.1

Research Subject Information and Consent Form

TITLE: DEVELOPMENT OF A NOVEL MODEL TO ASSESS QUALITATIVE AND QUANTITATIVE INJECTABLE DELIVERY DYNAMICS

VCU IRB Protocol # HM13424

VCU Clinical Research Study # 11EPHD

PRINCIPAL INVESTIGATORS:

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FOR GENERAL RESEARCH PROBLEMS CONTACT THE STUDENT

INVESTIGATOR:

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FOR MEDICAL EMERGENCIES CONTACT THE MEDICAL MONITOR:

John N. Clore, MD

VCU Medical Center

Department of Internal Medicine

Division of Endocrinology

1200 East Broad Street

P.O. Box 980155

Richmond, VA 23298

This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision. Reading this form and talking to the study doctor or study staff may help you decide whether to take part or not. If you decide to take part in this study, you must sign your name at the end of this form. Nothing can be done for the research study until you sign this form.

PURPOSE OF THE STUDY

The purpose of this research study is to make a model that shows where drugs go in your body after they are injected under your skin. You are being asked to participate in this study because you are a healthy volunteer, meet the study entry requirements, and are interested in being in the study.

Taking part in this study is entirely voluntary.

DESCRIPTION OF THE STUDY

This study involves receiving injections of a drug typically administered for viewing parts of the body using two different injectable delivery systems and receiving a computed tomography scan (CT scan) to verify where each of the delivery systems administers the drug. Iohexol is approved by the FDA for use in imaging different parts of the body. The drug is not typically used in the way that it will be used in this research and is, therefore, experimental. These drugs will be administered into your outer thigh, under the skin using two different delivery systems. One delivery system is a standard FDA-approved syringe and needle and the other is an FDA-approved automatic injector. The automatic injector is indicated for use with the approved syringe.

Your participation in this study may last up to 8 weeks. Up to 12 subjects will participate in this study.

Significant new findings developed during the course of the research that includes adverse reactions to the study drug or devices which may relate to your willingness to continue participation will be provided to you.

1. OVERVIEW OF PROCEDURES

If you decide to take part in this study, you will have a Screening Visit to see if you qualify. After the Screening Visit, there are 2 study periods, each consisting of 2 days and 1 night where you will stay in the clinic.

This is a single-blind study, therefore your study doctor and staff will know exactly whether you will be receiving the injection of the study drug by either the syringe and needle or auto-injector device. You will be prevented from seeing which delivery system (auto-injector or pre-filled syringe) is being used during your injections by placing a screen above your waist that prevents viewing of the injection area.

During Study Period 1, you will be randomly assigned (like the flip of a coin) to be given the study drug by using either the syringe or the auto-injector during the first period of the study. You have an equal chance of being assigned to either of the groups. Using the randomly assigned delivery system, the study drug, iohexol, will be given into your outer thigh, followed by a computed tomography (CT) scan to verify the location of injection. During Study Period 2, you will be given the study drug, iohexol, by using the other delivery system that was not used during the first study period.

You will not know which study drug delivery device is being used to give you the medicine. This is done (blinding) so that a fair evaluation of results may be made.

You will undergo a venous blood draw at least 10 times during each study period. On the day you are given the study drug, you will have a catheter (flexible tube) inserted into a vein to make multiple blood collections easier. The blood will be taken from this tube at different times, beginning just before dosing and throughout the course of the study following the CT scanning. Blood may have to be taken via needle sticks into your veins. In addition, you will be asked to provide a urine sample at least 5 times during each study period.

YOUR ROLE IN THE STUDY

Taking part in a research study can be an inconvenience to your daily life. Please consider the study time commitments and responsibilities as a research subject when you are deciding to take part. Your responsibilities as a study subject include the following:

- Tell the truth about your medical history and current conditions.
- Tell the study doctor if you have been in a research study in the last 30 days or are in another research study now.
- Do not donate blood for at least 30 days before taking study drug, throughout the entire study, and for 2 weeks after you receive the study drug.
- Tell the study doctor about any problems you have during the study.
- Be willing to fast at certain times during the study and follow meal and liquids requirements and restrictions for the study.
- Do not drink any alcohol for 24 hours before taking the study drug and until after collection of the final blood sample of the In-House Study Visit.
- Do not use tobacco (smoking cigarettes, pipes, cigars, chewing tobacco, nicotine patch, or any other nicotine containing products) for at least 24 hours before the Screening Visit and throughout the study.
- Do not take any prescription or non-prescription medicines, vitamins, dietary or herbal supplements throughout the study.
- From the time of your first dose of study medication until at least 2 weeks after completing the study, use at least two forms of contraception (e.g., condom plus IUD,

condom plus birth control pills, diaphragm with spermicide) if engaging in sexual intercourse where you or your partner could become pregnant.
- Do not take illegal drugs throughout the study.
- Bring enough money for travel to and from the study site.

Study Period 2 will take place at least 7 days after Visit 1. Therefore, your part in this study is expected to last about 4-6 weeks (possible time period from screening visit through the last study visit). There is no final follow-up visit for this study. Up to 12 subjects will take part in this study.

WHAT WILL HAPPEN DURING THE STUDY

Screening Visit

During the Screening Visit, the following procedures will be done:

- You will be asked about your medical history and about any medicines you are taking.
- You will have a physical exam.
- Your vital signs (heart rate, breathing rate, blood pressure and temperature), height, and weight will be measured. Your blood pressure will be measured in a lying down (supine) position.
- You will have an electrocardiogram (ECG – a tracing of the electrical activity of the heart).
- You will give blood for routine lab tests. Approximately 1-2 tablespoons of blood will be collected.
- Women will have a urine pregnancy test

If you qualify and wish to continue, Study Period 1 will be scheduled within 7 days after you complete this Screening Visit.

In-House Study Visits

Study Period 1 – Study Day 1 (Admission)

On this study day, the following procedures will be done:

- You will be asked about any changes in your health since the screening visit.
- You will be asked if you have taken any medicines, including prescription and over-the-counter medicines, vitamins, and herbal supplements, since the Screening Visit.
- Your vital signs (heart rate, breathing rate, blood pressure and temperature), height, and weight will be measured. Your blood pressure will be measured in a lying down (supine) position.
- You will be asked if you have used any drugs or alcohol in the last 24 hours.
- Women will have a pregnancy test.

If you continue to qualify, you will remain at the study site and will wait to receive your dose of drug. You will enter the unit at least 3 hours prior to your scheduled dosing time. If you have not already done so, you will be asked to fast for 4 hours.

Study Period 1 – Study Day 1 (Dosing)

The following procedures will occur after you have fasted:

- You will be asked how you are feeling.
- You will have a brief physical exam.
- You will have a catheter (flexible tube) inserted into a vein to make multiple blood collections easier.
    - You will give blood before receiving your injection and 15, 30, 40 minutes and 1, 1.5, 2, 4, 6, 8 and 10 hrs after dosing (for a total blood collection volume of approximately 60 cc or 4 tablespoons), to test for the amount of study drug in your blood.
- You will be asked to provide a urine sample before receiving your injection as well as 0-2, 2-3, 3-4, 4-6, 6-8 and 8-24 hours after dosing, to test for the amount of study drug in your urine.
- Your vital signs will be measured before dosing. Your blood pressure will be measured in a lying down (supine) position.
- You will then have a computed tomography (CT) scan of your thighs done below your pelvis.
- You will be given the dose of study drug (iohexol) using the randomly selected delivery system.
- You will then have additional computed tomography (CT) scan of your thighs done below your pelvis at 0.5, 10, and 20 minutes following the injection of the drug. You will be asked to remain lying in bed or sitting for at least 6 hours after you are given the study drug.

You will be given a standard meal at least two hours after taking the study drug. You will also be given water or other non-caffeinated beverages to drink after being given the study drug in order to ensure adequate urine production for collecting samples of the injected drug in your urine.

You will be discharged from the study site after remaining overnight in the unit at least 24 hours on Study Day 2, once all procedures are completed and the study doctor has decided you can leave. You will be scheduled to return to the study site at least 7 days later to begin Study Period 2.

Study Period 2 – Study Day 8 or more (Admission to the Site)

On this study day, the following procedures will be done:

- You will be asked about any changes in your health since the screening visit.
- You will be asked if you have taken any medicines, including prescription and over-the-counter medicines, vitamins, and herbal supplements, since the Screening Visit.
- Your vital signs (heart rate, breathing rate, blood pressure and temperature), height, and weight will be measured. Your blood pressure will be measured in a lying down (supine) position.
- You will be asked if you have used any drugs or alcohol in the last 24 hours.
- Women will have a pregnancy test.

If you continue to qualify, you will remain at the study site and will wait to receive your dose of drug. You will enter the unit at least 3 hours prior to your scheduled dosing time. If you have not already done so, you will be asked to fast for 4 hours.

Study Period 2 – Study Day 8 or more (Dosing Day & Discharge From Site)

The following procedures will occur after you have fasted:

- You will be asked how you are feeling.

- You will have a brief physical exam.
- You will have a catheter (flexible tube) inserted into a vein to make multiple blood collections easier.
    - You will give blood before receiving your injection and 15, 30, 40 minutes and 1, 1.5, 2, 4, 6, 8 and 10 hrs (for a total blood collection volume of approximately 60 cc or 4 tablespoons), to test for the amount of study drug in your blood.
- You will be asked to provide a urine sample before receiving your injection as well 0-2, 2-3, 3-4, 4-6, 6-8 and 8-24 hours after dosing, to test for the amount of study drug in your urine.
- Your vital signs will be measured before dosing. Your blood pressure will be measured in a lying down (supine) position.
- You will then have a computed tomography (CT) scan of your thighs done below your pelvis.
- You will be given the dose of study drug (iohexol) using the randomly selected delivery system.
- You will then have additional computed tomography (CT) scan of your thighs done below your pelvis at 0.5, 10, and 20 minutes following the injection of the drug. You will be asked to remain lying in bed or sitting for at least 6 hours after you are given the study drug.

You will be given a standard meal at least two hours after taking the study drug. You will also be given water or other non-caffeinated beverages to drink after being given the study drug. You will remain in the unit overnight until at least 24 hours following your administration of the study drug.

You will be discharged from the clinical unit once all procedures are completed and the study doctor has decided you can leave. Your participation in the study will be complete at this point.

RISKS AND DISCOMFORTS

Adverse reactions following the use of Iohexol are usually transient (not lasting for a long period of time), and are usually of mild to moderate severity, occurring in less than 5% of patients when administered in the vein.

However, serious, life-threatening and fatal reactions, mostly of cardiovascular origin (from the heart and blood vessels), have been associated with the administration of iodine-containing contrast media, including iohexol. These are rare in occurrence (<0.3%).

It should be noted that there is limited information on the safety of iohexol when injected into muscle or fat tissue beneath the skin. The amount of Iohexol that will be injected is less than a quarter of a teaspoon of fluid.

The injection of contrast media is frequently associated with the sensation of warmth and pain, especially in studies that look at arteries or veins using contrast media. Pain and warmth are less frequent and less severe with iohexol than with many contrast media.

Possible additional side effects associated with the use of iohexol include (by organ system):

Cardiovascular System:

- Arrhythmias (irregularity of heart rate)
- Angina/chest pain
- Low Blood Pressure

Nervous System:

- Vertigo (including dizziness and lightheadedness)
- Pain
- Vision abnormalities (including blurred vision)
- Headache
- Taste changes
- Rarely: Anxiety, fever, motor and speech problems, convulsions, paresthesia (numbness), somnolence (sleepiness), stiff neck, hemiparesis (numbness or inability to control one side of your body), syncope (fainting), shivering, transient ischemic attack (mini-stroke), cerebral infarction (stroke), and nystagmus (involuntary eye movement) have been reported, with an individual incidence of 0.3% or less.

Respiratory System:

- Rarely: Dyspnea (difficulty breathing), rhinitis (inflammation of the nasal passages), coughing, and laryngitis (inflammation of the throat), with an individual incidence of 0.2% or less.

Gastrointestinal System: Nausea and vomiting

- Others including diarrhea, indigestion, cramp, and dry mouth were reported, with an individual incidence of less than 0.1%.

Skin and Appendages:

- Hives, rashes, itching or other reactions of the skin with an individual incidence of less than 0.3%.

As the study procedures and study drugs might injure an unborn child, pregnant women may not participate in this study. Women who might become pregnant should use a medically accepted form of birth control such as total abstinence, birth control pills, an IUD, diaphragm, progesterone injections or implants, or condoms plus a spermicide. Methods of birth control other than total abstinence are not 100% effective, and should a woman become pregnant there is a risk of injury to an unborn child. For similar reasons, women who are nursing an infant may not participate.

Radiation Risks

As a participant in this study you will receive radiation exposure that is for research purposes only (not for your direct health benefit). Your radiation dose from participating in this study is approximately 1.3% of the annual permissible occupational exposure level for radiation workers.

The National Council on Radiation Protection and Measurements has set permissible occupational radiation exposure limits for many radiologists, technologists, and scientists who work with radiation and are exposed nearly every day. These limits are defined as the dose of radiation that, in light of present knowledge, is not expected to cause appreciable bodily injury to a person at any time during his/her lifetime. The risk of this amount of occupational exposure to radiation is, thus, considered to be very small. The radiation dose mentioned is what you receive from the research component of this study only and does not include any exposure you may have received or will receive in the future from other tests.

Injection Site Reactions

You might experience localized reactions that are from the injection or delivery system. Previous adverse events associated with the auto-injector included local injection site reactions that were mild and temporary, including bleeding, bruising, pain, redness, and swelling. In addition, although the injection is intended to be in your subcutaneous (fat) tissue, there is a possibility that the injection may occur in the muscle tissue that may or may not lead to localized reactions. Previous studies have demonstrated reactions that do occur with non-ionic contrast media, such as iohexol, injected into the subcutaneous (fat) or muscle tissue are localized to the injected area and are usually mild and temporary.

Unknown Risks

You might have side effects or discomforts that are not listed in this form. Some side effects may not be known yet. New ones could happen to you. Tell the study doctor or study staff right away if you have any problems.

BENEFITS TO YOU AND OTHERS

There is no guarantee that you will receive any medical benefits from being in this study.

This is not a treatment study, and you are not expected to receive any direct medical benefits from your participation in the study. The information from this research study may lead to a better understanding of how to give injectable drugs to patients outside of the hospital, which may also have an impact on treatments in the future for people with a variety of diseases. You may benefit from the physical exams, ECGs, lab tests, and other study procedures.

COSTS

There are no costs to you for participating in this study other than the time you spend in the study. This will be about an hour today, two days (including an overnight stay) for Study Period 1 and two days (including an overnight stay) for Study Period 2.

PAYMENT FOR PARTICIPATION

At the completion of each treatment period you will receive $150.00. If you complete both periods, you will receive an additional $100.00 for a total compensation of $400.00.

ALTERNATIVE TREATMENT

Since you are a healthy volunteer, your alternative is not to take part in this study.

CONFIDENTIALITY

Potentially identifiable information about you may consist of data abstracted from your medical screening log. Data is being collected only for research purposes. Your data will be identified by ID numbers, not names, and stored separately from medical records in a locked research area. All personal identifying information will be kept in password-protected files and these files will be deleted within 6 months from the completion of the study.

Other records including data obtained from the medical history and physical exam will be kept in a locked file cabinet. Access to all data will be limited to study personnel.

You should know that research data or (medical information if applicable) about you may be reviewed or copied by approved personnel of Virginia Commonwealth University. Personal information about you might be shared with or copied by authorized officials of the Department of Health and Human Services (if applicable).

What we find from this study may be presented at meetings or published in papers, but your name will never be used in these presentations of papers.

COMPENSATION FOR INJURY

Virginia Commonwealth University and the VCU Health System have no plan for providing long-term care or compensation in the event that you suffer injury as a result of your participation in this research study.

If you are injured or if you become ill as a result of your participation in this study, contact your study doctor immediately. Your study doctor will arrange for short-term emergency care or referral if it is needed.

Fees for such treatment may be billed to you or to appropriate third party insurance. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study.

VOLUNTARY PARTICIPATION AND WITHDRAWAL

Your participation in this study is voluntary. You may decide to not participate in this study. Your decision not to take part will not result in a penalty or loss of benefits to which you are otherwise entitled. If you do participate, you may freely withdraw from the study at any time.

Your participation in this study may be stopped at any time by the study doctor without your consent. The reasons might include:

· the study doctor thinks it necessary for your health or safety;

· you have not followed study instructions;

· the study has been stopped; or

· administrative reasons require your withdrawal.

If you stop participating in the study before it is finished, you should complete the final study visit so that the study team can assess your health and follow up on any continuing adverse events.

GETTING ANSWERS TO YOUR QUESTIONS ABOUT THE STUDY

You can ask questions about this consent form or the study before you decide to start the study or at any time during the study.

Contact the study doctor or study staff with any questions or concerns. Their telephone numbers are printed on the first page of this form.

If you have questions about your rights as a research subject, you may contact:

Office of Research

Virginia Commonwealth University

800 East Leigh Street, Suite 113

PO Box 980568

Richmond, VA 23298

(804) 827-2157

You may also contact this number for general questions, concerns or complaints about the research. Please call this number if you cannot reach the research team or wish to talk to someone else.

Do not sign this consent form unless you have had a chance to ask questions and have had all of your questions answered. Additional information about participation in research studies can be found at http://www.research.vcu.edu/irb/volunteers.htm.

1. CONSENT

I have been provided with an opportunity to read this consent form carefully. All of the questions that I have about this study have been answered.

By signing this consent form, I have not waived any of the legal rights or benefits, to which I otherwise would be entitled. My signature indicates that I freely consent to participate in this research study. I will receive a copy of the consent form once I have agreed to participate.

_____

Subject Name, printed

_____ _____

Subject Signature Date

_____ _____

Signature of Person Conducting Consent Date

_____ _____

Printed Name of Person Conducting Consent Date

_____ _____

Witness Signature Date

_____ _____

Printed Name of Witness Date

_____ _____

Principal Investigator Signature (if different from above) Date

APPENDIX B.2

Informed Consent Process Documentation Form

Subject Initials _____ Subject Number:_____

Informed Consent Process Documentation

Please **INITIAL** next by **"yes" or "no"** on each line as appropriate **(if no, you MUST explain in the notes section below):**

_____ Yes _____ No Subject was given a copy of the ICF to read.

_____ Yes _____ No Ample time was given to the subject to read and ask questions.

_____ Yes _____ No All questions and concerns were addressed prior to signing consent form.

_____ Yes _____ No A copy of the consent form was provided to the subject.

_____ Yes _____ No No study procedures were performed prior to signing of the consent form.

_____ Yes _____ No Was an Assent required.

Who was present during the ICF process?

_____

The details of this research study were discussed with the subject. The study was explained in detail including all the contents of the informed consent document. The patient/subject was encouraged to ask questions. All questions were answered to the satisfaction of the patient/subject. The patient/subject was given adequate time to read the informed consent form and the opportunity to discuss it. The patient demonstrated understanding of the informed consent document and indicated that they would like to participate in the study. The patient demonstrated understanding that this is a research study. The IRB-approved informed consent document was signed without alteration by the patient/subject. A copy of the informed consent document was placed in the patient/subject record, and a copy was given to the patient/subject. No activities specifically related to research were started until after the execution of the consent.

The subject signed informed consent document, approval date:

Yes OR NO (circle one) on _____.

_____ _____

Signature of person that obtained consent Date

Appendix C.1

William H. Barr Curriculum Vitae

Summary of Qualifications (CV)

WILLIAM BARR, PHARM D, PHD

Virginia Commonwealth University

School of Pharmacy-Center For Drug Studies

MCV West Hospital; Room 12-410

1200 E. Broad St

Richmond VA 23298

Business Phone Number – 804-828-8334

Fax-804-828-6902

EDUCATION:

B.S. Pharmacy (highest honors) - Univ. Calif. (S.F.) – 1960

Pharm.D. – Univ. Calif. (S.F.) – 1961

Ph.D. (Pharmaceutical Chemistry) – Univ. Calif. (S.F.) – 1966

(Pharmacology minor)

TRAINING:

Clinical research beginning in 1964, graduate thesis (UCSF)

Asst/Assoc Professor of Pharmaceutics and Research Asst/Assoc Professor of Pediatrics (clinical research in Pediatrics and Clinical Pharmacokinetics), (SUNY @ Buffalo), 1966-1972

Professor and Chairman, Dept. of Pharmacy and Pharmaceutics, Virginia Commonwealth University/Medical College of Virginia, 1972-2001.

Director, Clinical Research Unit (Center for Drug Studies) for 20 years, (VCU) 1982-present.

Has been Principal Investigator on over 90 clinical studies (Phase 1)

RELEVANT AFFILIATIONS: Professor, School of Pharmacy, VCU

Director, Center for Drug Studies, VCU

MAJOR OR RELEVANT PUBLICATIONS:

Over 150 publications (chapters, papers, and abstracts) relating to biopharmaceutics, clinical pharmacokinetics and clinical pharmacology.

APPENDIX C.2

Curriculum Vitae

WILLIAM RUSSELL GARNETT, PHARM.D.

ADDRESS

VA Commonwealth University
Medical College of Virginia
410 N. 12th Street, Rm. 334
Box 980533
Richmond, VA 23298-0533 (mail)
23219 (Fed-Ex)

Telephone: 804-828-8328
FAX: 804-828-8359
e-mail: WRGarnet@VCU.EDU

BIRTH DATE AND PLACE

June 2, 1946
Farmville, VA

EDUCATION

Hampden-Sydney College	1964-1966
Hampden-Sydney VA	
Pre-pharmacy	
Medical College of VA	1966-1969
Richmond, VA	

School of Pharmacy

B.S. Pharmacy

Philadelphia College of Pharmacy and Science 1971-1973

Philadelphia, Pennsylvania

Doctor of Pharmacy

ACADEMIC POSITIONS

Instructor in Clinical Pharmacy July 1, 1973-June 30, 1976

University of NC at Chapel Hill

School of Pharmacy

Chapel Hill, NC

VA Commonwealth University

Medical College of VA

School of Pharmacy

Department of Pharmacy

Richmond, VA

Assistant Professor July 1, 1976 - June 30, 1981

Associate Professor (tenure) July 1, 1981 - June 30, 1986

Professor (tenure) July 1, 1986 - present

Graduate Faculty July 1989 - present

Interim Chairman April 1998 – 1999

COMMITTEES - MCV/VCU

Multiple, including:

Pharm.D. Admissions Committee - School of Pharmacy, MCV, 1978 - 1986

Pharm.D. Promotion Committee - School of Pharmacy, MCV, 1976 - present

School of Pharmacy B.S. Curriculum Committee, 1982 - 1986

Graduate Program Committee - 1988 - present

Interdisciplinary Research Grant Committee, 1998 to present

Non-Traditional Pharm.D. Program Committee, 1998 to present

MCV Hospital Pharmacy - Certification of Clinical Competency Committee, 1981-1985

MCVH Pharmacy and Therapeutics Committee, 1982-1985

MCV/VCU Institutional Research Committee, 1982-1985

VCU Promotion and Tenure Appeal Committee - 1990-1993, Chairman 1991-1992

LICENSURE AS A PHARMACIST

Commonwealth of VA Licensure #004233 (June 1969)

SELECT MEMBERSHIPS

American Society of Hospital Pharmacists

American Pharmaceutical Association

Rho Chi Honorary Pharmaceutical Society

Sigma Zeta Honorary Science Society

American Association of Colleges of Pharmacy

VA Pharmaceutical Association

American Association of Pharmaceutical Sciences

American College of Clinical Pharmacy

Phi Kappa Phi Honor Society (inducted as a faculty member)

RESEARCH ADVISOR - Research Committee for 7 Ph.D. Students

HONORS:

- Alpha Delta Chapter of Phi Delta Chi - Alumni of the Year
- Selected the Robert Leonard Memorial Lecturer, TX Society of Hospital Pharmacist, 1993
- Phi Kappa Phi Honor Society (faculty member)
- Elected Fellow American College of Clinical Pharmacy, 1993
- American College of Clinical Pharmacy Education Award, 1996
- Invited Professor to Tan Tock Seng by Singapore Ministry of Health 8-96 to 9-96

SELECT PUBLICATIONS IN REFERRED JOURNALS

- Garnett, William R.: "Diluents for Antineoplastic Drugs," Drug Intelligence and Clinical Pharmacy 5:261 (Aug)1971.

- Garnett, William R. and Snyder, Thomas C.: "Indomethacin's Value," J. Am. Pharm. Assoc., NS13(2):66 (Feb) 1973. - Garnett, William R.; Carter, Barry L.; and Pellock, John M.:"Bioavailability of Phenytoin Administered with Antacids," Therapeutic Drug Monitoring, Vol. 1, 1979, p. 435-437.

- Garnett, William R.; Carter, Barry L.; and Pellock, John M.: "Effect of Calcium and Antacids on Phenytoin Bioavailability,: Arch Neurol. Vol. 37, (July) 1980, p. 467.

- Garnett, William R.; Goldberg, Jeffrey A.; and Lowenthal, Werner: "Evaluation of a Systemic Approach to Clinical Pharmacy Consultation in a Long Term Care Facility," The Gerontologist. Vol. 21 No. 2 (April), 1981, p.151-157.

- Garnett, William R.; Davis, Larry J.; McKenney, James M.; Steiner, Kenneth C.: "Evaluation of the Effect of a Follow-Up Telephone Call on Patient Compliance," Am. J. Hosp. Pharm. Vol. 38, No. 5,(May), 1981, p.676-679.

- Garnett, William R.: "Sucralfate - An Alternative in Peptic Ulcer Therapy," Clin. Pharm. Vol. 1, No.4, July-Aug. 1982, 307-314.

- Garnett, William R.: "Discontinuing Anticonvulsant Medications," Clin. Pharm. Vol. 3, No. 4, Sept. - Sept-Oct., 1984, p. 456-457.

- Garnett, William R.: "Mechanism of Action of Drugs Used in the Treatment of Drug Induced Gastritis," Practical Gastroenterology. Vol. X No. 5 Sept/Oct 1986 p.40-44.

- Garnett, William R.: "Is The Clinical Scientist an Oxymoron?" Am J Pharm Ed. Vol. 52, Winter 1988, p467-468.

- Garnett, William R.: "The Final Frontier: Clinical Pharmacy Practice in Community Pharmacy Settings." Am J Pharm Educ. Vol. 53, Fall 1989, p313-314.

- Garnett William R.: "Geriatric Pharmacokinetics," FL J Hosp Pharm. Vol. 9, 1989, p23-28.

- Garnett, William R.: Patient Outcome Management of Acid Related Disorders. J Am Soc Consulting Pharm. 1992.

- Garnett, William R.: Epilepsy. US Pharmacist. December 1992.

- Garnett, William R.: Efficacy, safety, and cost issues in managing patients with gastroesophageal reflux disease. Am J Hospital Pharm. 1993; 50(Suppl 1):S11-S18. - Garnett William R.: Fluvastatin cost considerations. Ann Pharmacotherapy. 1994; 28:1111-1112.

- Garnett William R.: Chronopharmacology: Giving the right drug at the right time. Clinical Trends in Pharmacy Practice. 1994; 8:16-28.

- Garnett William R.: The pharmacology of fluvastatin, a new HMG-CoA reductase inhibitor. Clin Cardiol. 1994; 17(Suppl IV):3-10.

- Garnett William R. and Pellock JM: AFocus on lamotrigine: A new antiepileptic drug for patients with partial seizures. Hosp Formul. 1994; 29:806-812.

- Garnett William R.: New opportunities for the treatment of epilepsy. Am. J. Health - Syst Pharm. 1995; 52, 88-91.

- Garnett William R and Pellock JM: ACritical drug appraisal: Lamotrigine - Effective oral add-on therapy. P & T. 1995; 20:156-170.

- Garnett William R.: Drug interactions with the HMG-CoA reductase inhibitors.@ Am J Health-Sys Pharm. 1995, 52; 1639-45.

- Garnett William R.: Lansoprazole. Ann pharmacotherapy. 1996; 30: 1425-1436

- Garnett William R: Treatment and prevention of acid-related disorders. US Pharmacist. 1997; 22:119-130.

- Garnett WR, Levy B, McLean AM, et al. Pharmacokinetic evaluation of twice-daily extended-release carbamazepine (CBZ and four-times daily immediate release CBZ in patients with epilepsy. *Epilepsia.* 1998 Mar;39(3): 274 – 9.

- Garnett WR. Antiepileptic drug treatment: outcomes and adherence. *Pharmacotherapy.* 2000 Aug;20(8 Pt 2): 191S – 199S.

- Garnett WR, Yunker NS. Treatment of Crohn's disease with infliximab. *Am J Health Syst Pharm.* 2001;Feb 15;58(4): 307 – 16.

- Garnett WR. Clinical implications of drug interactions with coxibs. *Pharmacotherapy.* 2001; Oct;21(10)1223 -32.

In addition, Dr. W.R. Garnett has been involved in over ten book reviews, authored 50 book chapters and published over 45 abstracts, and led over 775 presentations during his academic career.

APPENDIX C.3

John N. Clore M.S., M.D. Curriculum Vitae

1 BIOGRAPHICAL SKETCH

NAME Clore, John Newton	POSITION TITLE Professor of Medicine, VCU
eRA COMMONS USER NAME jnclore	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Virginia Commonwealth University, Richmond VA	BS	1976	Biology
Virginia Commonwealth University, Richmond VA	MS	1977	Biology
Virginia Commonwealth University, Richmond VA	MD	1982	Medicine

Virginia Commonwealth University, Richmond VA	Resident	1982-1985	Internal Medicine
Virginia Commonwealth University, Richmond VA	Fellow	1985-1988	Endocrinology

A. Personal Statement

In my role as Program Director of the General Clinical Research center at VCU for more than 15 years, I have had the opportunity to mentor a number of young investigators who have gone on to academic careers focused in clinical research. This role has included instruction in methodology as well as critique and assistance in data analysis, manuscript writing and submission of grants. Recognition of my abilities as a mentor include my selection as Distinguished Mentor in Clinical Science by the School of Medicine at VCU. My particular area of clinical and research interest for over 25 years has focused on diabetes mellitus, the focus of the present application. I have an active clinical practice which will enhance the candidate's ability to recruit potential research subjects and I will be able to assist the candidate in clinical outcomes measures.

B. Positions and Honors.

Professional Positions:

Virginia Commonwealth University (VCU)

1988-1993 Assistant Professor of Medicine

1993-1998 Associate Professor of Medicine

1996- Program Director, General Clinical Research Center

1998- Professor of Medicine

1998- Professor of Biochemistry and Molecular Biophysics

2007- Associate Vice President for Clinical Research

Selected Honors:

AOA, William Harrison Higgins Award,1985;

Young Investigator Award, ADA, 1987

Clinical Associate Physician Award, NIH, 1987-1990.

Distinguished Mentor in Clinical Science, 2008

C. Selected Peer-Reviewed Publications

1**. Clore JN** and Blackard WG (1994). Suppression of gluconeogenesis does not deplete liver glycogen in patients with NIDDM after a three-day fast. Diabetes 43: 256-262, 1994.

2. **Clore JN,** Li J, Gill R, Gupta S, Zuelzer W and Blackard WG. (1998) Skeletal Muscle Phosphatidylcholine Fatty Acids and Insulin Sensitivity in Normal Man. Am J Physiol 275: E665-E670.

3. **Clore JN,** Li J, Zuelzer W, Harris P and Rizzo W. (1999) Changes in Skeletal Muscle Phosphatidylcholine Fatty Acid Composition Alters Insulin Responsiveness in Normal Man. Metabolism 49:232-238.

4**. Clore JN,** Stillman J and Sugerman HJ. (2000) Glucose-6-phosphatase flux is increased in Type 2 diabetes. Diabetes 49:969-974

5. Sugerman HJ, Wolfe LG, Sica DA and **Clore JN**. Diabetes and hypertension in severe obesity and effects of gastric bypass-induced weight loss. Annals of Surgery 237:751-758, 2003.

6. Levy JR, **Clore JN** and Stevens W. Dietary n-3 Polyunsaturated Fatty Acids Decrease Hepatic Triglycerides, Hepatology 39:608-616. 2004.

7. **Clore JN,** Levy JR and Stillman JS. Acute infusion of saturated but not polyunsaturated fatty acids impairs hepatic insulin action in man. Am J Physiol 287:E358-E365, 2004.

8. Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling AK, Stravitz RT, Shiffman ML, **Clore JN**, Mills AS. A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol.

2:1107-15, 2004

9. Shannon, K.A., R.M. Shannon, **Clore JN**, C. Gennings, B.J. Warren, and J.A. Potteiger. Resistance exercise and postprandial lipemia: an investigation into the dose effect of differing volumes of acute resistance exercise bouts. Metabolism: Clinical and Experimental, 54:756-763 2005

10. Shannon KA, Shannon RM, **Clore JN**, Gennings C, Warren BJ, Potteiger JA. Effects of acute aerobic exercise on postprandial lipemia: a comparative investigation of untrained African American and Caucasian women. International Journal of Sport Nutrition & Exercise Metabolism 18:37-48, 2008

11. Luebbers PE, Potteiger JA,Warren BJ,**Clore JN**,Gennings C, Bond DS. Glucose Uptake after Resistance

12. Shannon KA, Shannon RM, **Clore JN**, Gennings C, Warren BJ, Potteiger JA. Effects of acute aerobic exercise on postprandial lipemia: a comparative investigation of untrained African American and Caucasian women. International Journal of Sport Nutrition & Exercise Metabolism 18:37-48, 2008

D. Research Support

Ongoing Research Support

1UL1RR031990-01 (Clore JN, PI). 07/01/10-06/30/15

Institutional Clinical and Translational Science Award

The VCU CTSA provides broad-based infrastructure support for clinical and translational research across the institution.

Role: Principal Investigator

N66001-09-2-2060 (Cifu, PI) 05/01/2009-4/30/2011

Hyperbaric Oxygen Therapy (HBO2T) for Post-Concussive Symptoms (PCS) After Mild Traumatic Brain Injury (MTBI): A Randomized, Double-Blinded, Sham-Controlled, Variable Dose, Prospective Trial.

This trial will assess the efficacy of hyperbaric oxygen in veterans with traumatic brain injury.

Role: Co-investigator,

Completed Research Support

Agency: National Center for Research Resources 9/01/06-8/31/07

Type P20 RR023414

This Planning Grant for an Institutional Clinical and Translational Science Award supports the establishment of a new Center for Translational Science and the development of degree programs in clinical and translational research.

Role: Principal Investigator

Type: U01 DK61731

"The role of de-novo lipogenesis in non-alcoholic fatty liver disease."

The long-term objective of this project is to examine the mechanisms by which fatty liver occurs in some but not all individuals with insulin resistance.

Role: Principal Investigator

Agency: National Institutes of Diabetes, Clore (PI) 6/01/1996-6/30/2001

Digestive Diseases and Kidney

Type: RO1 DK43013,

"Control Mechanisms of Hepatic Glucose Output"

The long-term objective of this project is to examine the mechanisms by which hepatic glucose production is increased in patients with Type 2 diabetes mellitus. Particular emphasis has been placed on the reciprocal relationship between liver glycogen release and gluconeogenesis which is stimulated by free fatty acids.

Role: Principal Investigator

APPENDIX D

Randomization Schedule

Virginia Commonwealth University
Protocol # 11EPHD / IRB # HM13424

Randomization Worksheet

SUBJECT NUMBER	TREATMENT	RANDOMIZATION NUMBER
1	AP	304
2	PA	312
3	PA	315
4	AP	314
5	PA	317
6	AP	318
7	PA	307
8	AP	306
9	PA	309
10	AP	311
11	PA	310
12	AP	313

AP = Autoinjector in Period 1 and Prefilled Syringe in Period 2
PA = Prefilled Syringe in Period 1 and Autoinjector in Period 2

APPENDIX E.1a

Interim Study Flowsheet

Protocol # 13424 PIs: Garnett, W., Barr, W., & Edwards, E.

Development of a novel model to assess qualitative & quantitative injectable delivery dynamics

□ Study Period 1 □ Study Period 2 Date:_____ Unit Arrival Time:_____

ID#_____ HT _____cm WT _____ kg □ Confirm Fasting x4hr:

Yes____No____

□ Review Exclusion Criteria (Study Questionnaire) □ Have Pt change/dress

□ Place Saline lock (Use Catheter Tracking Log) □ Quick Vue Urine Pregnancy Test (females):_____

□ 45-60 Minutes prior to Dosing – obtain pre-dose Blood Draw, followed by Pre-dose Urine

□ VS(within 60 min predosing)Time: _____ BP(supine 5 min)__________ HR_____

RR_____ Temp _____C

□ Transport to Radiology 30 minutes prior to Scheduled Dosing Time

□ Pt. to remain sitting or lying in bed for 6 hours post dosing(Bath Room Privileges Only)

□ Encourage water and non-caffeinated beverages post dosing

Time	Rel Time	Actual Time	PK Lt GrnTT 4.5ml	**CT Scan**	Urine 2 ml	Staff Initials	Comments
Pre-injection			X		X TV:		
Pre-injection Scan				**X** Period 1 only			
Injection SubCut (0 min)	Prefilled syringe or Auto-injector Site: (Right or Left Thigh?_____@ _____				0-2hr TV:_____		
0.5 min (30 sec)				**X**	Actual Collection Time:_____		
2 min				**X**			
3 min.			X				
5 min				**X**			
7 min			X				
10 min				**x**			
12 min			X				
15 min				**X**			
20 min			X				
40 min			X				
1 hr			X				
2 hr (Meal)			X				
4 hr			X		2-4 hr TV: _____ Actual Collection Time		
6 hr			X		4-6hr TV: Actual Collection Time		
8 hr			X		6-24hr		
24 hr					TV: Actual Collection Time		
					BP (supine 5min)__________ HR_____ RR_____ Temp _____C		

Nurse Signature/initials: _____

APPENDIX E.1b

Final Study Flowsheet

Protocol # 13424 PIs: Garnett, W., Barr, W., & Edwards, E.

Development of a novel model to assess qualitative & quantitative injectable delivery dynamics

□ Study Period 1 □ Study Period 2 Date:_____ Unit Arrival Time:_____

ID#_____ HT _____cm WT _____ kg □ Fasting except water,etc x4hr (see order sheet): Yes____No____

□ Review Exclusion Criteria (Study Questionnaire) □ Pt to wear shorts □Place Saline Lock

□ Quick Vue Urine Pregnancy Test (females):_____ □ Urine and Lt GrnTT on wet ice/refrigerate during collection*

□ 45-60 Minutes prior to Dosing – obtain pre-dose Blood Draw, followed by Pre-dose Urine

□ VS (within 60 min predosing) Time: _____ BP(supine 5 min)__________ HR_____ RR_____ Temp _____C

□ Transport to Radiology 30 minutes prior to Scheduled Dosing Time

□ Pt to remain sitting or lying in bed for 6 hours post dosing (Bath Room Privileges Only)

□ Encourage water and non-caffeinated beverages post dosing

Time	Rel Time	Actual Time	PK Lt GrnTT* 4.5ml	**CT Scan**	Urine* 2 ml **(collection ends prior to blood draw)**	Staff Initials	Comments
Pre-injection			X		X (spot) TV:		
Pre-injection Scan				**X** Period 1 only			
Injection SubCut (0 min)	Prefilled syringe or Auto-injector (N) Site: (Right or Left Thigh? 1.0 ml @ _____				X 0-2hr TV:_____ Actual Collection Time:_____	Dose Check /	
30 sec				**X**			
15 min			X				
10 min				**X**			
20 min				**X**			
30 min			X				
40 min			X				
1 hr			X				
1.5 hr			X				
2 hr (Meal)			X		X 2-3 hr TV: _____ Actual Collection Time		
4hr			X		X 3-4 hr TV: _____ Actual Collection Time		
					X 4-6 hr TV: _____ Actual Collection Time		
6 hr			X				
					X 6-8 hr TV: _____ Actual Collection Time		
8 hr			X				
					X 8-24 hr TV: _____ Actual Collection Time	BP (supine 5min)__________ HR_____ RR_____ Temp _____C	
10 hr			X				
24 hr							

APPENDIX E.2

Adverse Event Reporting Form

ADVERSE EVENTS	**Subject Initials:** _____ **Subject Number:** _____	Page _____ of _____

Has the subject complained of any adverse event? Yes No ***If there are more than 4 AE's use another form.**

**** If YES, complete a SERIOUS ADVERSE EVENT form.**

Adverse Event* (one per line) Whenever possible, signs and symptoms should be grouped together as syndromes or diagnoses	Occurrence **Onset and Resolution Of Adverse Event** Date MM / DD / YY Time (24-hr Clock)	Is the adverse event serious? 1 = no 2 = **yes	Intensity 1 = Mild 2 = Moderate 3 = Severe 4= Life threatening 5= Death		Action Taken 1 = None 2 = Permanently discontinued from Study		Con Med Taken for this AE? 1= No 2 = Yes	Outcome 1 = Resolved without sequelae 2 = Resolved with sequelae 3= Not resolved 4= Death	**Relationship to study drug/device** 1= Possibly Related 2 = Probably Related 3= Not Related	**M.D. Initial and Date**
Staff Init. ___ date __ # ___ Staff Init _____ date _____	Onset ___/___/___ __:___ Resolved ___/___/___ ___:___		MD INIT.		MD INIT.		MD INIT.			

Staff Init. _____ date _____ # ___ Staff Init _____ date _____	Onset ___/___/___ :___ Resolution ___/___/___ ___:___		MD INIT.		MD INIT.		MD INIT.			
Staff Init. _____ date _____ # ___ Staff Init _____ date _____	Onset ___/___/___ ___:___ Resolution ___/___/___ ___:___		MD INIT.		MD INIT.		MD INIT.			
Staff Init _____ date _____ # ___ Staff Init _____ date _____	Onset ___/___/___ ___:___ Resolution ___/___/___ ___:___		MD INIT.		MD INIT.		MD INIT.			

Q. C. ON_____BY_____

Adverse Events Observation Form

Subject Initials: _____ Subject Number: _____

Event #	Entry Date	Entry Time	

Attach Additional Pages As Needed.

Page_____ of _____

Q. C. ON_____BY_____

Subject Initials: _____ Subject Number: _____

INSTRUCTIONS: ***Record Vital Signs according to investigator instructions if deemed necessary for an adverse event.***

Date	**Study Day**	**Actual Time** (24-hour clock)	**Blood Pressure (mmHg)**	**Pulse (bpm)**	**Respiratory Rate (per min)**	**Oral Temp (°F)**	**Staff Initials**	**M.D. Review**
			/					
			/					
			/					
			/					
			/					
			/					
			/					

APPENDIX E.3

Screening and Demographic Form

DOB: _____Screen Date: _____
Age: _____
Volunteer ID verified by: _____
Race: _____ Ethnicity: Hispanic: **Yes** ☐ **No** ☐ ID Document:_____
Sex: _____ Latino: **Yes** ☐ **No** ☐
Consent form Signed date:_____Signed time:_____

This is a confidential record. Information contained herein will not be released unless authorized by the patient. The volunteer has been given sufficient time to read, ask questions and sign the ICF prior to study related procedures being performed. The volunteer has been given a copy of the signed consent. Staff Init: _____ Time: _____ _____

VITAL SIGNS

Height	_____cm. / in.	Time: _____
Weight	_____kg. / lbs.	Time: _____
BMI:_____		
Oral Temperature	_____°F	Time: _____
Respiration Rate	_____/Min	Time: _____
Thigh Circumference: _____cm		Time: _____
Vital Signs (supine 5 min.) BP_____/ _____ P_____		Time: _____
Urine Pregnancy Quick Vue Results:_____		Time:_____
ECG: _____		Time:_____

Repeated: Yes ☐ **No** ☐

Yes ☐ **No** ☐ Oral Temperature _____°F Time: _____

Yes ☐ **No** ☐ Respiration Rate _____/Min Time: _____

Yes ☐ **No** ☐ Vital Signs Seated 10min. BP_____/ P_____ Time: _____

M.D.
Orders:_____
_____
_____
_____
_____

Date:_____

MD. Signature:_____
Nurse Initials/Signature:_____

APPENDIX E.4

Physical Examination Form

This is a confidential record. Information contained herein will not be released unless authorized by the patient.

Date: _____ **Subject Initials:** _____ **Subject Number:** _____

System	Normal	Abnormal	Not Done	Findings if Abnormal
General Appearance				
Skin				
EENT				
Head/Neck/Thyroid				
Lymphatic				
Lungs/Chest				
Cardiovascular				
Abdomen				
Genitourinary				
Extremities				
Neurological				
Musculoskeletal				
Other: _____				

Comments:

_____

_____

_____

Physician Signature: _____ **Date:** _____/ _____ / _____

Physician Printed Name: _____

APPENDIX E.5

Medical History Evaluation Form

Screening Date: _____Screening Number:_____Subject Initials:_____

MEDICAL HISTORY

This is a confidential record. Information contained herein will not be released unless authorized by the patient.

If the answer is Yes to any of the questions, please indicate the specific disease condition, the date of occurrence, the duration of the illness and any other information available.

Please initial and date the bottom of each page to indicate you have reviewed each **Condition** on the page.

Y or N	Condition
_____	**CARDIOVASCULAR** - Have you ever had any heart related diseases including angina, arrhythmia, congestive heart failure, hypertension, hypotension, myocardial infarction (heart attack), peripheral vascular disease, coronary artery bypass graft, angioplasty, hyperlipedemia or heart murmur?
_____	**GASTROINTESTINAL** - Have you ever had any diseases pertaining to the stomach or intestine including ulcerative colitis, Crohns disease, gastritis, ulcers, or hernias?
_____	**HEPATIC -** Have you ever had any liver related diseases (e.g. hepatitis or jaundice)?
_____	**ENDOCRINE** - Have you ever had any endocrine or metabolic diseases including diabetes (Type I or Type II), hyperthyroidism, or hypothyroidism?
_____	**GENITOURINARY** - Have you ever had any genitourinary diseases including impaired renal (kidney) function, pyelonephritis, kidney stones, kidney or bladder diseases? If female, have you had a hysterectomy?

_____ **HEMATOLOGICAL** - Have you ever had any blood related diseases including anemia or tendency to bleed (such as easy bruising or prolonged bleeding after tooth extraction, etc.)?

_____ **NEUROLOGICAL**- Have you ever had any neurological diseases including transient ischemic attach (a temporary interference with blood supply to the brain), CONVULSIONS, SEIZURES, STROKE, EPILEPSY, HEAD TRAUMA, INTRACRANIAL HEMMORRHAGE?

_____ **MUSCULOSKELETAL**- Have you ever had any musculoskeletal problems such as broken bones or diseases including arthritis or muscular dystrophy?

_____ **RESPIRATORY**- Have you ever had any respiratory or pulmonary diseases including asthma, bronchitis, or chronic obstructive lung disease?

_____ **IMMUNOLOGICAL**- Have you ever had any immunological diseases including, immunodeficiency, or connective tissue disease (such as generalized inflammation of connective tissue & blood vessels)?

_____ **NEOPLASTIC**- Have you ever had any neoplastic disease including cancer, Leukemia, or lymphoma?

_____ **DERMATOLOGICAL-** Have you ever had any problems related to the skin?

_____ **SERIOUS ILLNESS, INJURY, OR SURGERY**- Have you ever had any other serious illness, injury or surgery not yet reported?

_____ **EAR, EYES. NOSE, THROAT or MOUTH** - Have you had EENT procedures not previously reported (I.E. tonsillitis, tonsillectomy, adnoid problems, deviated septum, chronic dental problems etc.)?Do you wear eye glasses, contacts, dental work or hearing aid, etc?

_____**PSYCHIATRIC** - Have you ever been hospitalized for any psychiatric problems?

_____ **OTHER -** Have you ever had any other physical or medical conditions which have not been mentioned in the preceding questions?

_____ **MEDICATIONS -** Have you taken any medications in the past 30 days? Include all medications either prescription or over the counter (OTC), taken on a regular basis. Include any medications taken seasonally for allergies.

_____ **ALLERGIES**- Are you allergic to any drugs or medications, either Prescription or Over the Counter. This would include having a bad reaction to any drug or form of medicine.
Do you have any other allergies (Environmental, Food, Immunizations, Vitamins, or XRay dyes).

_____ **HIV**- Have you ever tested positive for the HIV virus? If yes, indicate the date you tested positive and any other pertinent information.

APPENDIX E.6

Personal Habits Form

The following questions concern your personal habits and other general information. If the answer is Yes to any of the questions, please fill out the information requested in the table that follows each question.
Please initial the bottom of each page to indicate you have reviewed each question on the page.

Yes/No **Condition**

_____ Do you currently use, or have you ever used any form of **TOBACCO** (cigarettes, cigars, pipe or chewing)?

Form	# per day	Unit	Start Date	Stop Date

_____ Do you drink **ALCOHOL**? If yes indicate the type (beer, wine, liquor), how much you drink per day, week or month and the most recent date of alcohol consumption.

Type	Amount	Per	Most recent	Detail Notes

_____ Have you ever been treated for **ALCOHOL or SUBSTANCE ABUSE**?

Date	Date Treated	Type	Where Treated	Detail Notes

_____ Have you used any **ILLICIT DRUGS** in the past 6 months?

If yes, please give date of last usage and what type (marijuana, cocaine, speed, acid etc.)

(PLEASE BE HONEST, WE MAY REQUIRE A URINE DRUG SCREEN. IF YOU SHOULD TEST POSITIVE FOR ANY ILLICIT DRUGS, YOU MAY BE DISQUALIFIED FOR FUTURE STUDIES WITH VCU.)

Date of last usage	Drug Type	Detail Notes

_____ Do you drink **CAFFEINATED BEVERAGES**? If yes indicate what type (Tea, Cola, Coffee), the amount per day, week, or month, and the most recent date of caffeine consumption.

Type	Amount	Per	Most recent	Detail Notes

_____ Are you a vegetarian or do you have special **DIET RESTRICTIONS** (due to allergies, etc.)?

Diet Restriction

_____ Do you have a **ROUTINE EXERCISE** program?

Type of Exercise	Frequency	Detail Notes

_____ If any, when was the last time you received, **donated or had a loss of blood or blood products**? Indicate type (whole blood, plasma, platelet), when and where.

Type	When	Where	Detail Notes

If any, when is the last time you participated in an experimental **DRUG or experimental MEDICAL DEVICE STUDY**?

Study Name	Completion Code	Last Dosing Date	Study Location

APPENDIX F

Iohexol Inter-run Precision and Accuracy Results for the Quality Controls and Back Calculated Values for the Calibration Standards

Back-Calculated Values for

Plasma Iohexol

	Iohexol Concentration (µg/mL)				
RUN	**2.50**	**10.00**	**25.00**	**50.00**	**100.00**
14 APR11 STD CTRL CHECK	A	9.98	25.42	48.52	96.87
	2.42	9.26	24.75	50.05	104.36
29 APR11 306 307 V2	2.11	A	24.44	53.13	101.88
	2.43	9.11	26.40	49.11	98.56
18 MAY11 V1 10 SUBJ	2.51	11.48	27.88	60.23	60.23
	2.82	9.38	25.65	52.82	109.03
20MAY11 V2 10 SUBJ	2.24	9.54	24.71	48.43	95.54
	2.81	10.09	26.63	45.62	93.34
08 JUN11 V1 AND V2 10 SUBJ	2.47	9.91	28.96	48.31	88.65
	2.49	9.92	26.29	50.65	99.21
Mean	2.5	9.9	26.1	50.7	94.8
StdDev	0.23	0.70	1.5	4.0	13.4
%RSD	9.3	7.1	5.6	7.9	14.1
%DFN	-1.0	-1.5	4.5	1.4	-5.2

A = deleted from calculations per SOP criteria of ± 15%

Back-Calculated Values for

Urine Iohexol

	Iohexol concentration (µg/mL)				
RUN	**2.50**	**10.00**	**25.00**	**50.00**	**100.00**
25 APR11 306 307 PU	A	10.6	26.0	53.9	93.1
	2.4	10.2	25.2	50.3	88.0
25 APR11 306 307 URINE	2.6	A	28.6	54.4	108.8
	A	7.3	23.3	48.4	99.6
30 APR11 306 307 URINEV2	2.3	10.2	25.6	51.9	96.2
	A	14.5	25.0	45.0	102.1
Mean	2.45	10.57	25.60	50.65	97.95
StdDev	0.19	2.56	1.72	3.54	7.27
%RSD	7.7	24.2	6.7	7.0	7.4
%DFN	-2.2	5.7	2.4	1.3	-2.0

A = deleted from calculations per SOP criteria of ± 15%

Plasma Inter-run Quality Control

RUN		Iohexol concentration (µg/mL) 5.00	30.00	75.00
08 JUN11 V1 AND V2 10 SUBJ		6.0	28.2	75.8
		5.7	29.1	75.8
14 APR11 STD CTRL CHECK		4.6	30.0	74.4
		5.2	25.4	73.5
		5.6	26.1	66.7
18 MAY11 V1 10 SUBJ		5.2	18.8	44.9
		4.4	13.4	69.9
20MAY11 V2 10 SUBJ		4.0	20.5	68.8
		4.4	28.6	78.0
29 APR11 306 307 V2		4.6	30.3	78.8
		4.4	33.8	76.0
	Mean	4.9	25.8	71.1
	StdDev	0.65	6.0	9.5
	%RSD	13.2	23.2	13.3
	%DFN	-1.7	-13.9	-5.1

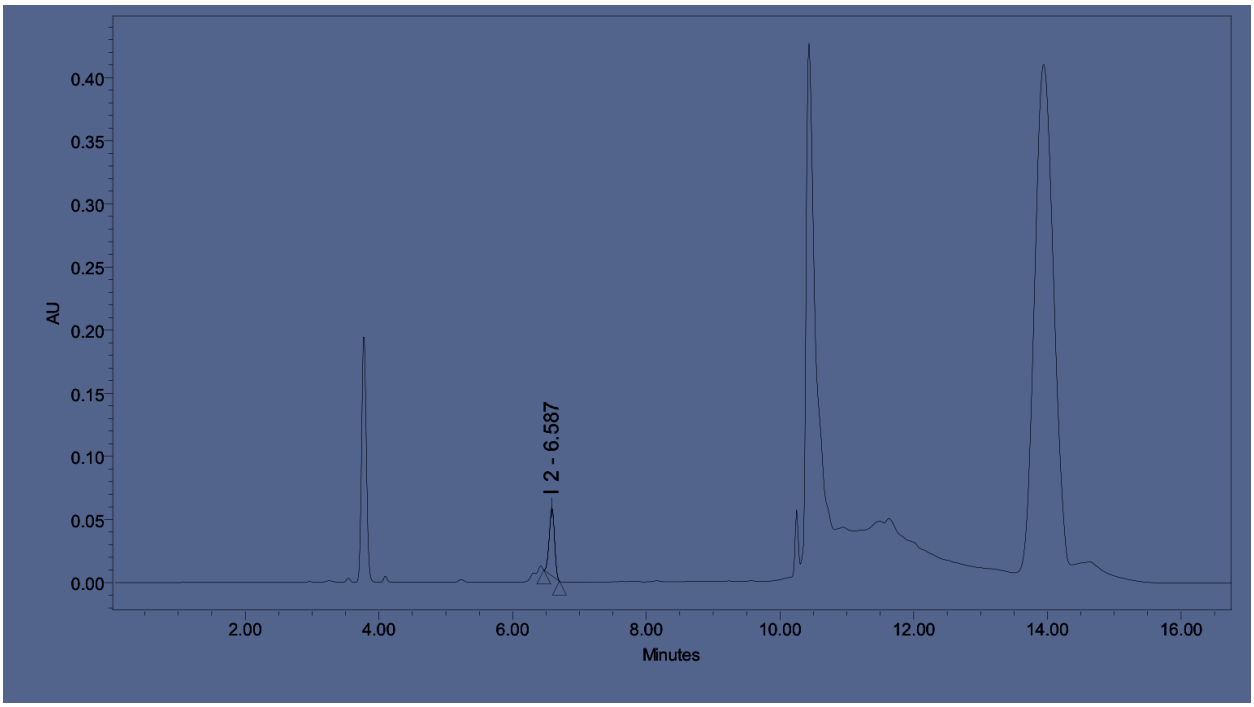
Urine Inter-run Quality Control

RUN		Iohexol concentration (µg/mL) 5.00	30.00	75.00
25 APR11 306 307 PU		5.3	22.7	81.9
		5.2	30.3	79.4
25 APR11 306 307 URINE		5.9	24.8	64.5
		5.1	25.7	68.5
30 APR11 306 307 URINEV2		5.1	33.2	77.1
		4.9	28.9	75.9
	Mean	5.21	27.93	74.62
	StdDev	0.33	3.66	6.09
	%RSD	6.3	13.1	8.2
	%DFN	4.3	-6.9	-0.5

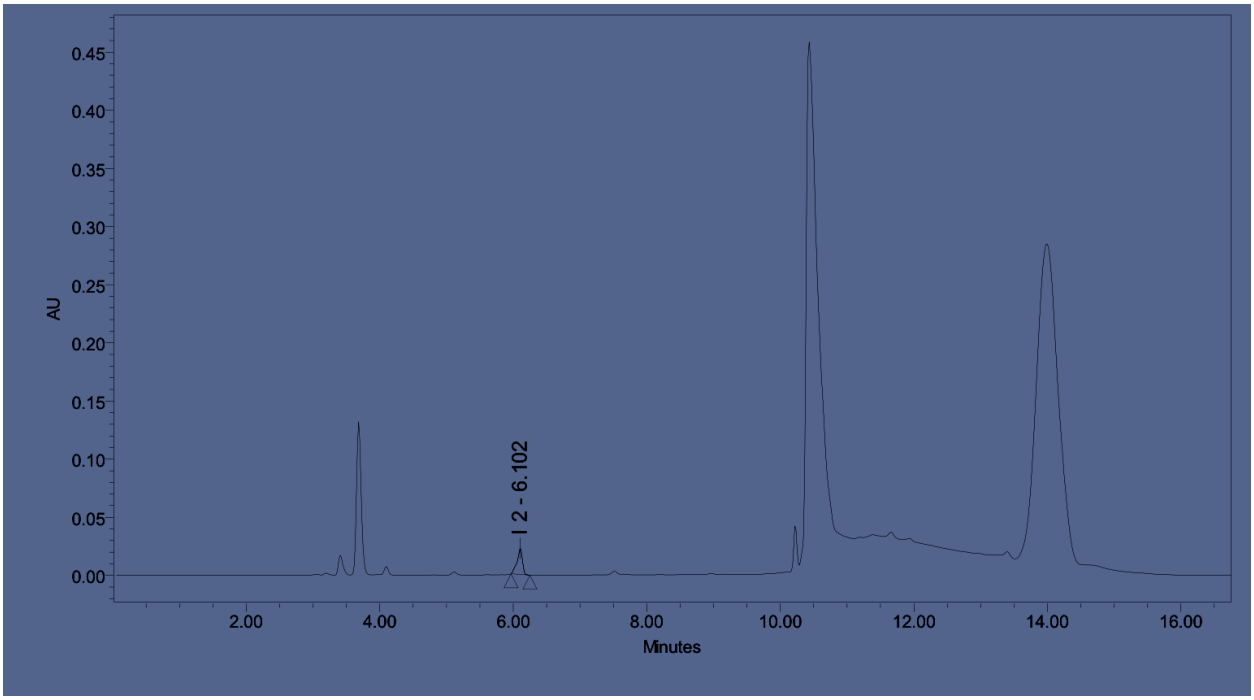
APPENDIX G

Sample Chromatograms

Sample Chromatogram: Calibration Standard 3 (Note: I2 was used for quantification)



Sample Chromatogram: Sample 307-Period 2- Sampling Time - 2 hours



APPENDIX H.1

Demographics – Continuous Variables

		Treatment Sequence		
	Statistic	AP N = 6	PA N = 6	Total N = 12
Age (years)	N	6	6	12
	Mean	27.17	33.0	30.08
	SD	8.30	12.85	10.76
	Median	24	27.5	24
	Min	22	22	22
	Max	44	51	51
Height (cm)	N	6	6	12
	Mean	174.88	173.10	173.99
	SD	8.25	11.53	9.60
	Median	177.05	171.25	174.55
	Min	161.7	160.1	160.1
	Max	184.6	187.5	187.5
Weight (kg)	N	6	6	12
	Mean	69.9	68.4	69.15
	SD	8.46	13.44	10.73
	Median	69.45	65.3	68.05
	Min	58.9	55.0	55.0
	Max	80.4	90.0	90.0
BMI (kg/m^2)	N	6	6	12
	Mean	22.82	22.59	22.70
	SD	1.83	1.77	1.72
	Median	22.65	21.81	21.9
	Min	20.64	21.45	20.64
	Max	25.13	26.01	26.01
Thigh circumference (cm)	N	6	6	12
	Mean	47.21	51.72	49.47
	SD	13.09	3.15	9.38
	Median	50.7	51.0	50.7
	Min	21.1	48.5	21.1
	Max	57.5	57.5	57.5

A= Auto-injector, P= Pre-filled Syringe

APPENDIX H.2

Demographics – Categorical Variables

	Treatment Sequence		
	AP N = 6	PA N = 6	Total N = 12
Race			
Black or African American	2 (33.3%)	2 (33.3%)	4 (33.3%)
Asian	1 (16.7%)	0 (0.0%)	1 (8.3%)
White	3 (50.0%)	4 (66.6%)	7 (58.3%)
American Indian or Alaska Native	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gender			
Female	3 (50.0%)	3 (50.0%)	6 (50.0%)
Male	3 (50.0%)	3 (50.0%)	6 (50.0%)

A= Auto-injector, P= Pre-filled Syringe

APPENDIX I

Assumptions Utilized for the Calculation of Lamda z (λz)

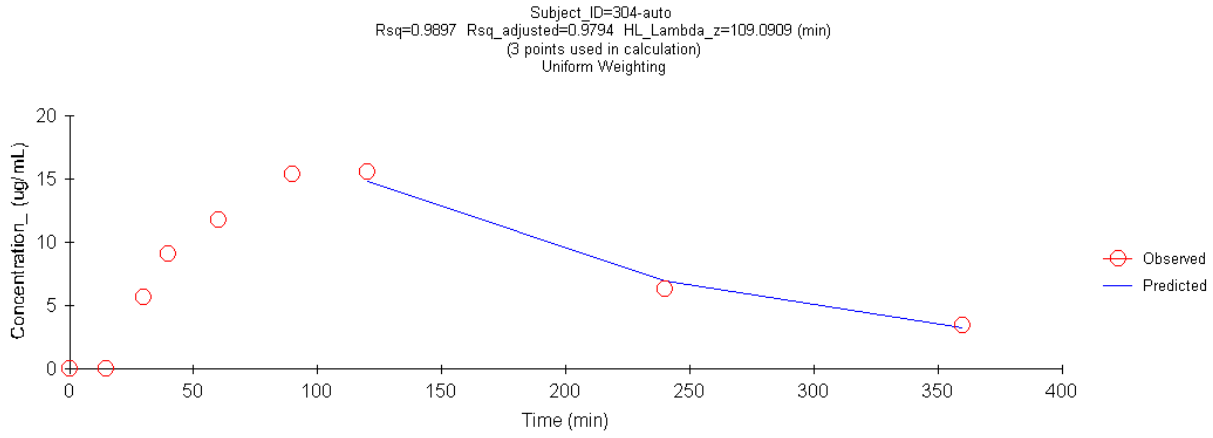
Terminal Elimination Rate Constant Data Point Range Selection by Subject

Subject #	Auto-injector	Pre-filled Syringe
304	last 3 points (C_{max} included)	last 3 points
309	acceptable as run	last 4 points
310	last 4 points included	no terminal phase - excluded
311	last 3 points included (except C_{max})	acceptable as run
312	acceptable as run	last 3 points
313	No terminal phase - excluded	acceptable as run
314	last 3 points (C_{max} included)	acceptable as run
315	acceptable as run	acceptable as run
317	last 3 points included (except C_{max})	acceptable as run
318	last 3 points (C_{max} included)	last 3 points (C_{max} included)

APPENDIX J

Individual Subject Plasma Concentration-Time Profiles for the Auto-injector and Pre-filled Syringe Treatment Groups (Linear and Semi-log Scales) by Subject

Treatment Group: Auto-Injector



X vs. Observed Y and Predicted Y

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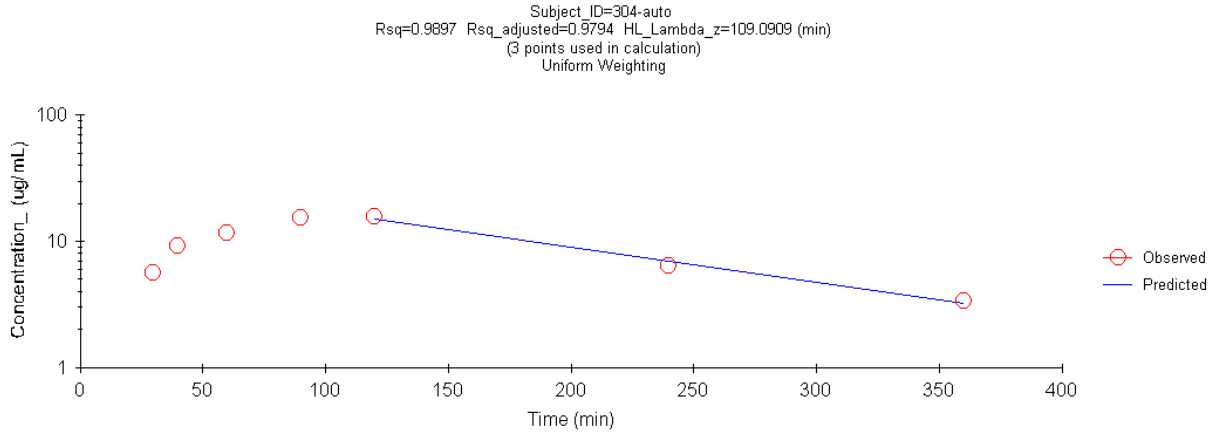

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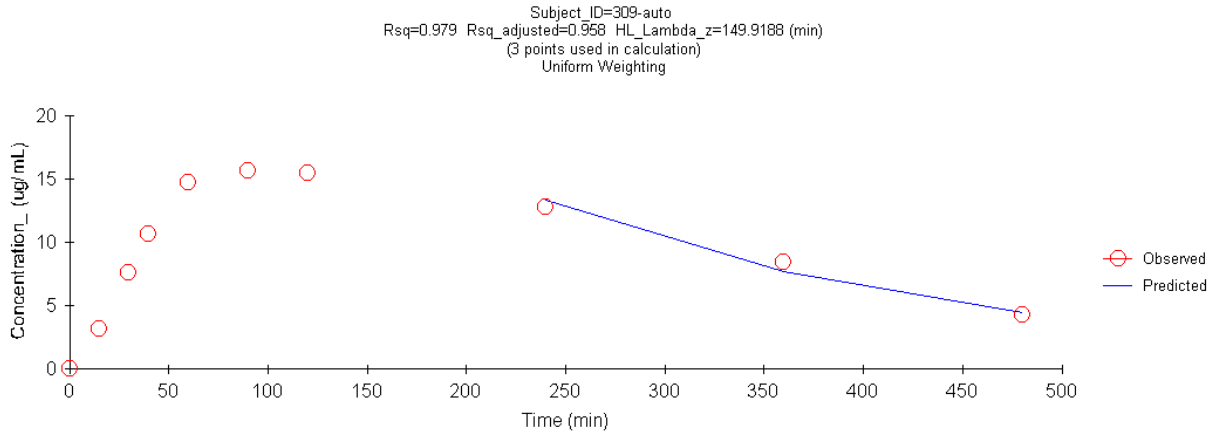

X vs. Observed Y and Predicted Y

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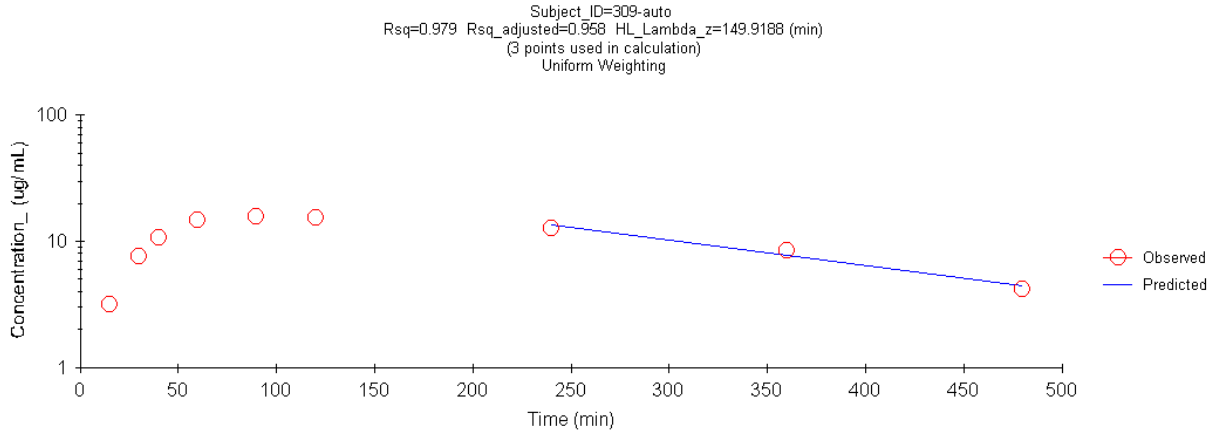

X vs. Observed Y and Predicted Y

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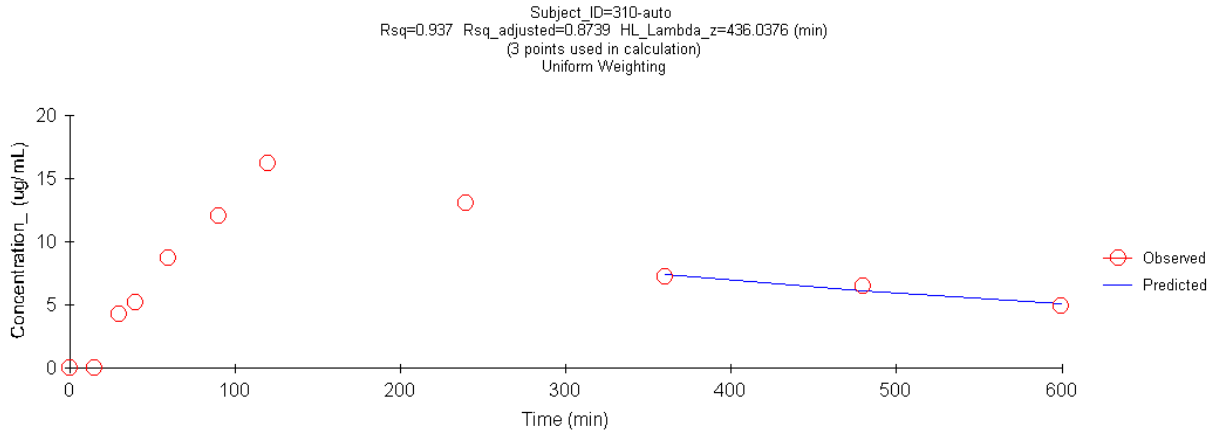

X vs. Observed Y and Predicted Y

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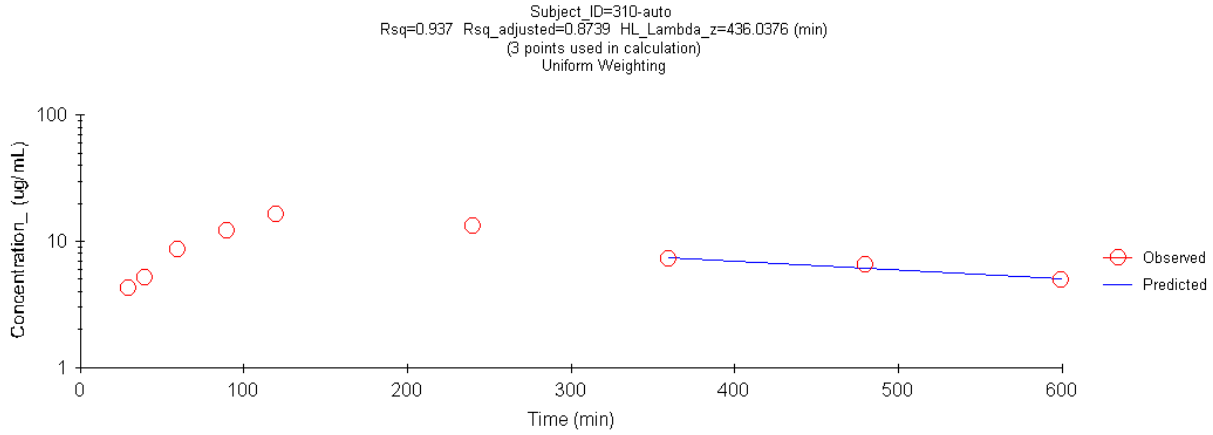

X vs. Observed Y and Predicted Y

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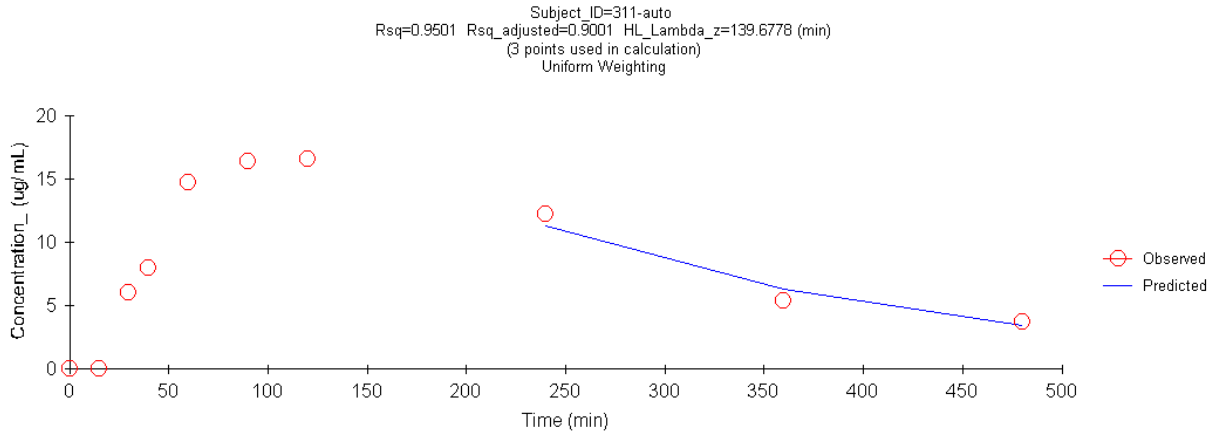

X vs. Observed Y and Predicted Y

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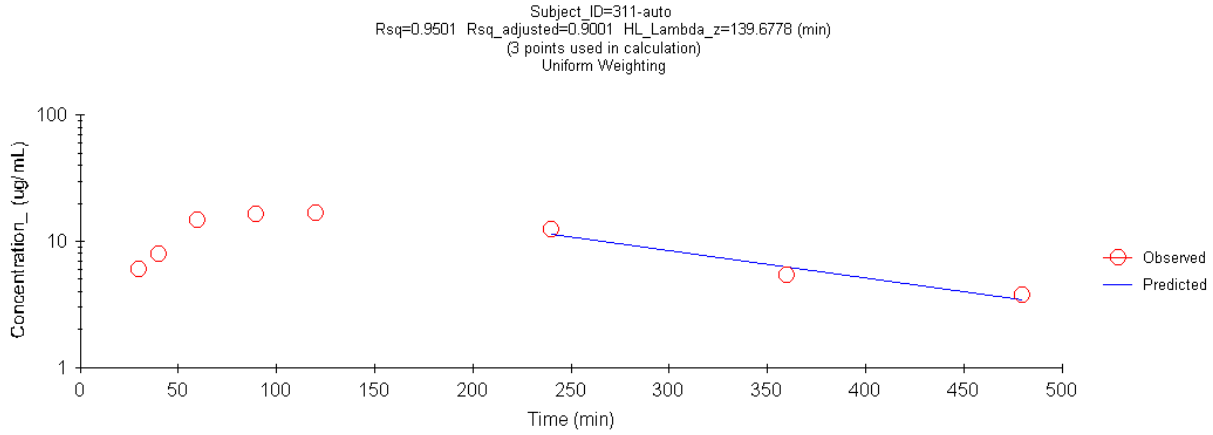

X vs. Observed Y and Predicted Y

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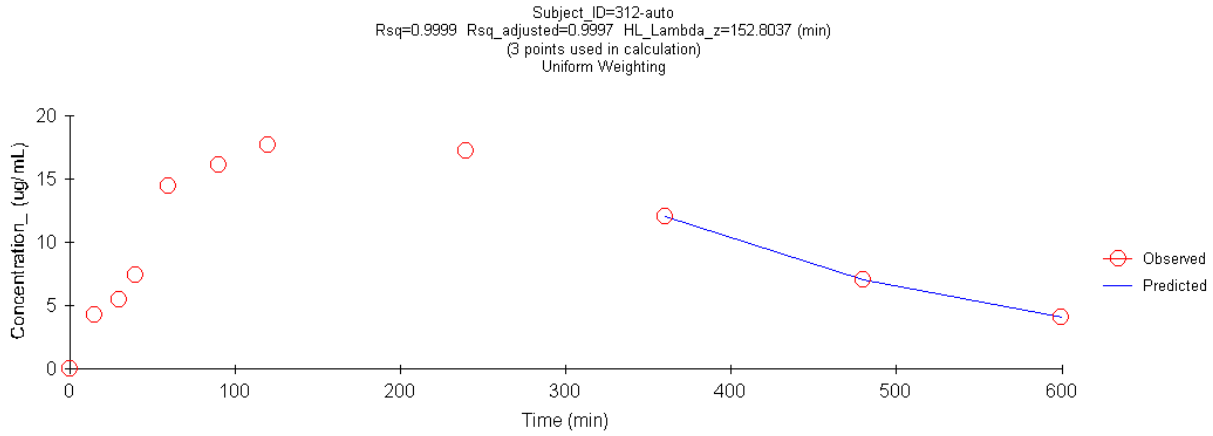

X vs. Observed Y and Predicted Y

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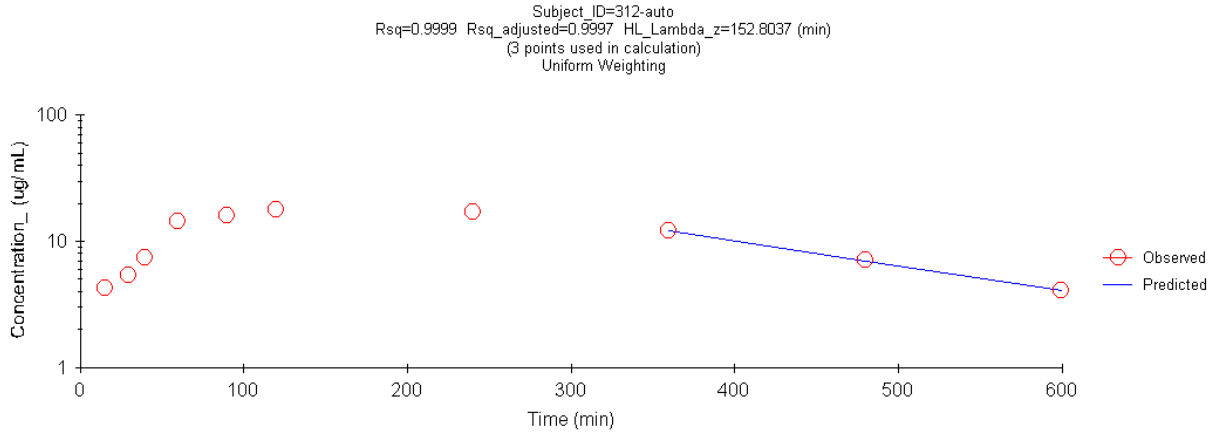

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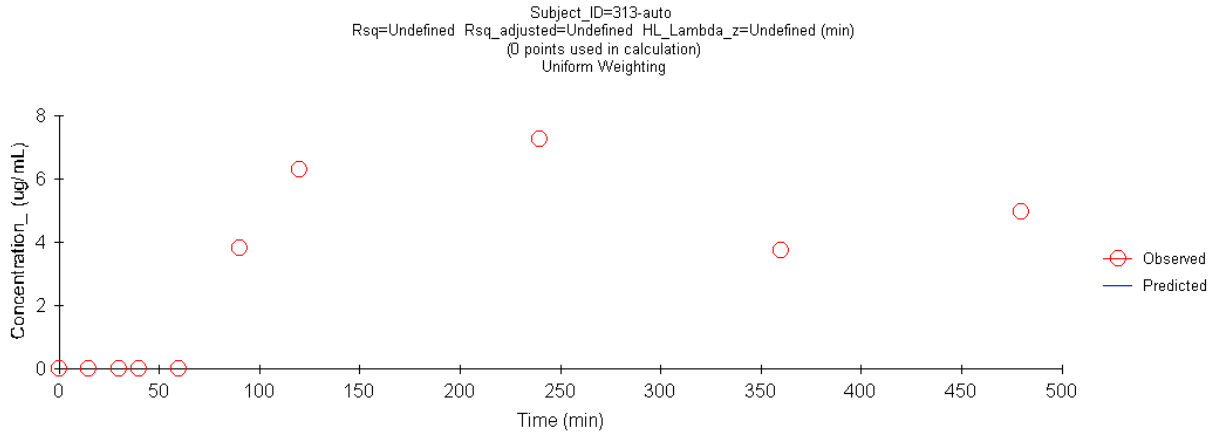

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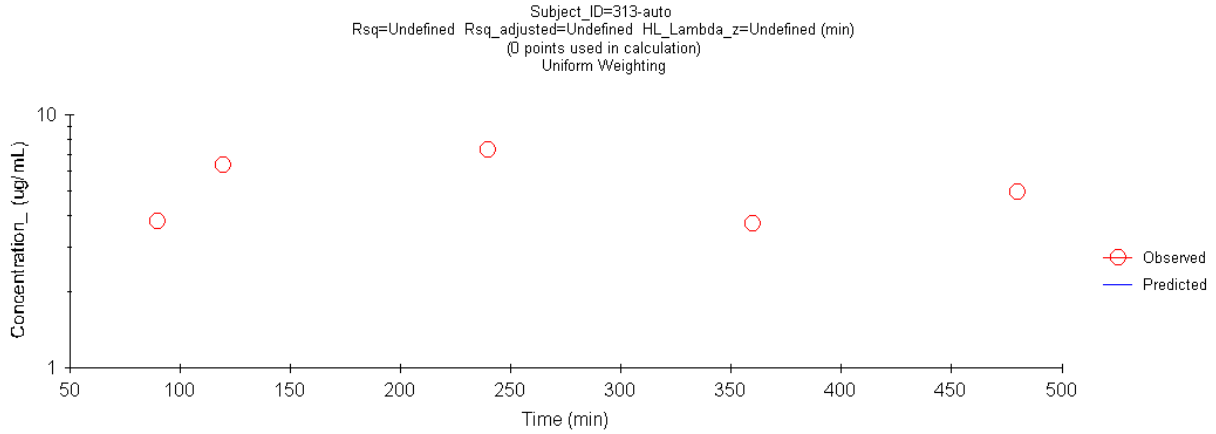
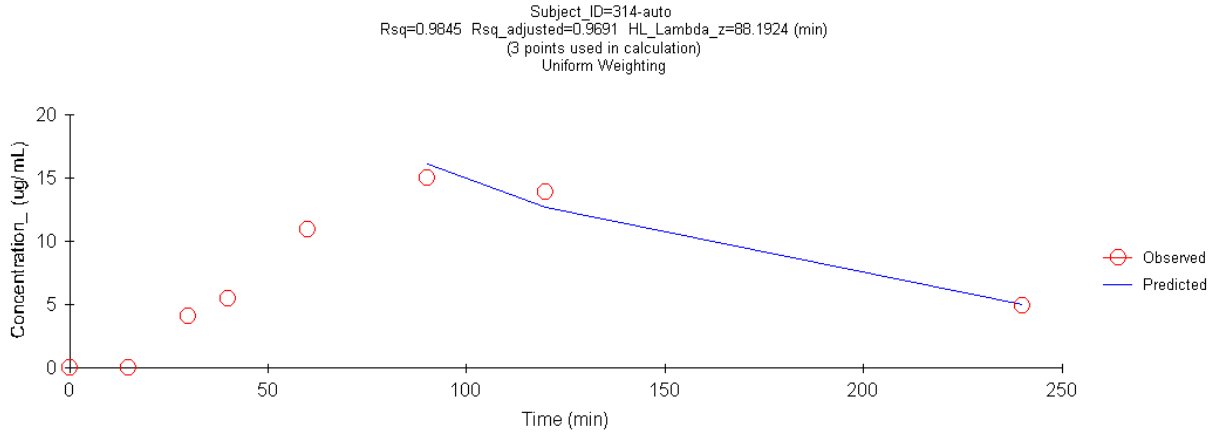


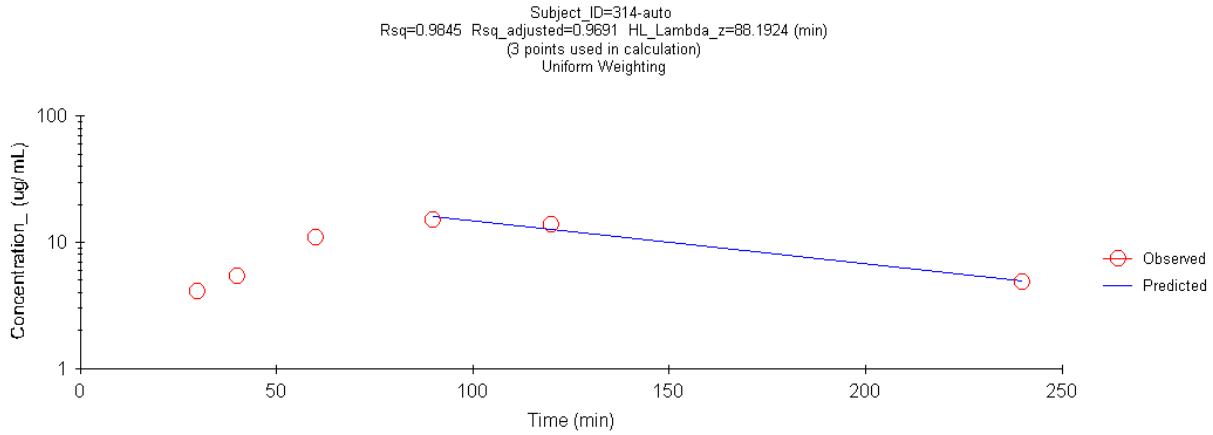
X vs. Observed Y and Predicted Y

C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



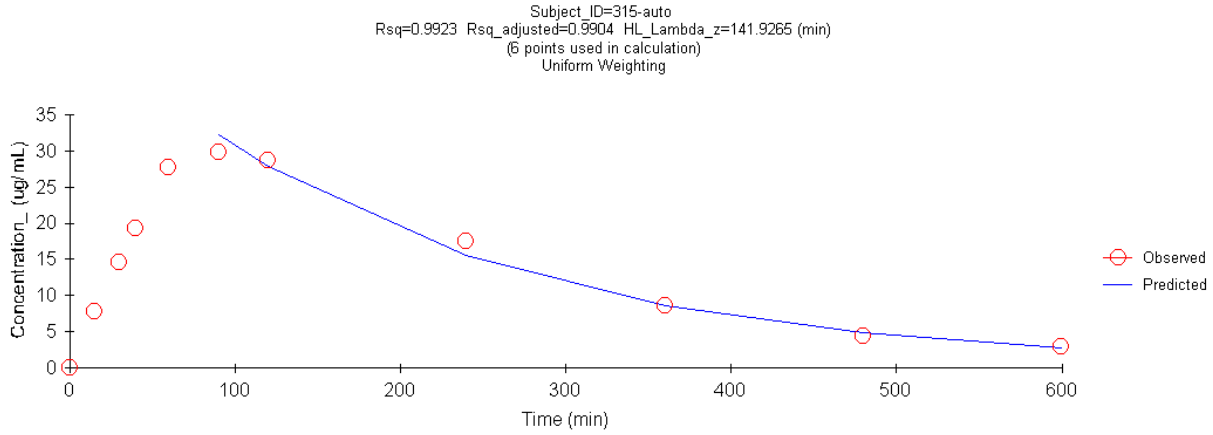
X vs. Observed Y and Predicted Y

C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



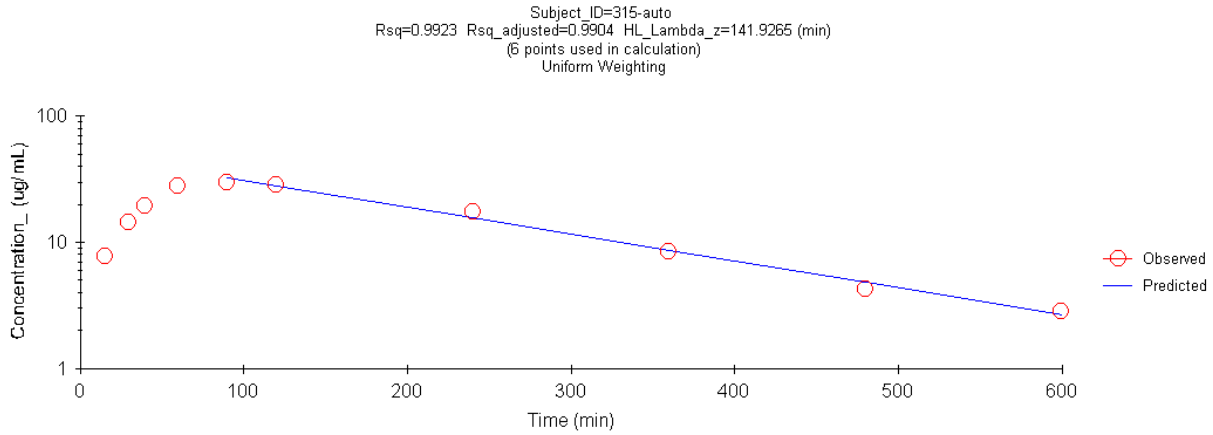
X vs. Observed Y and Predicted Y

C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



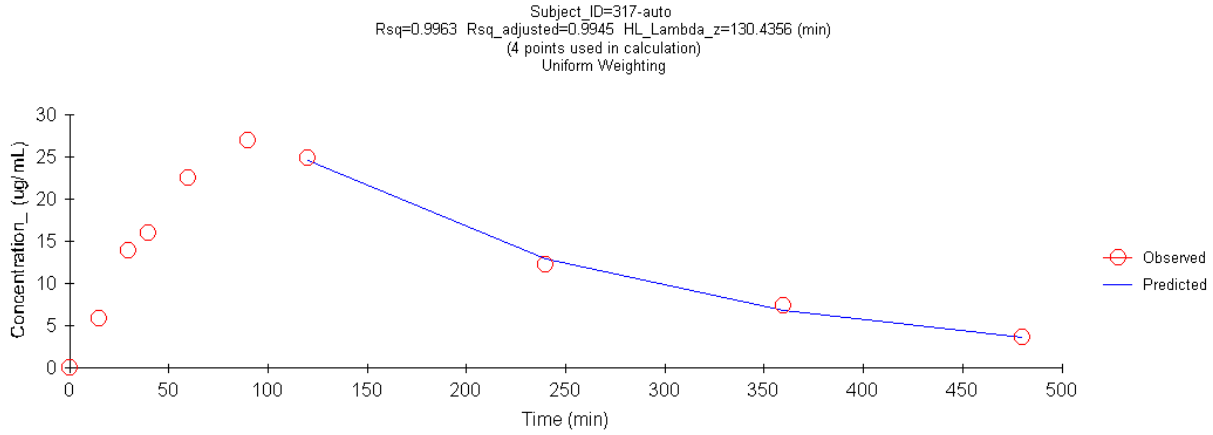
X vs. Observed Y and Predicted Y

C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



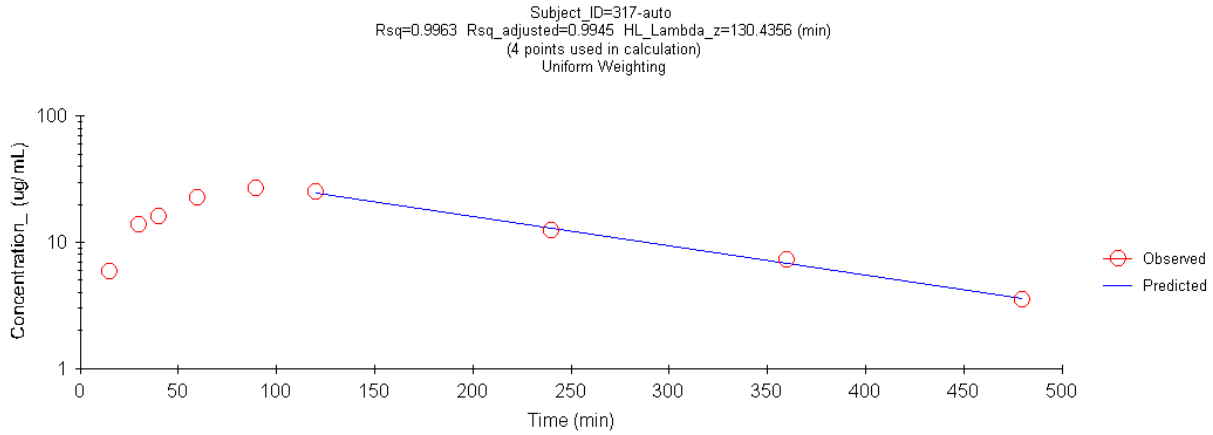
X vs. Observed Y and Predicted Y

C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



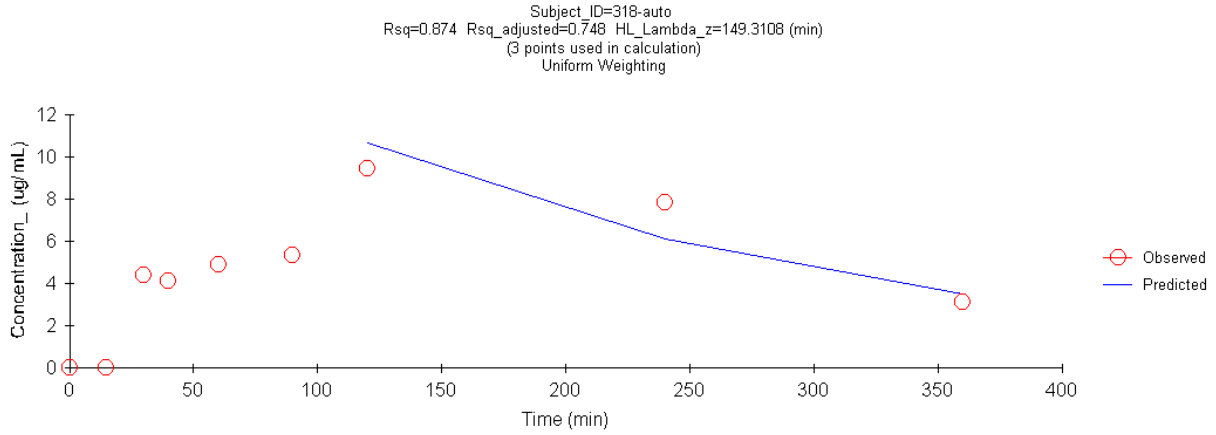
X vs. Observed Y and Predicted Y

C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



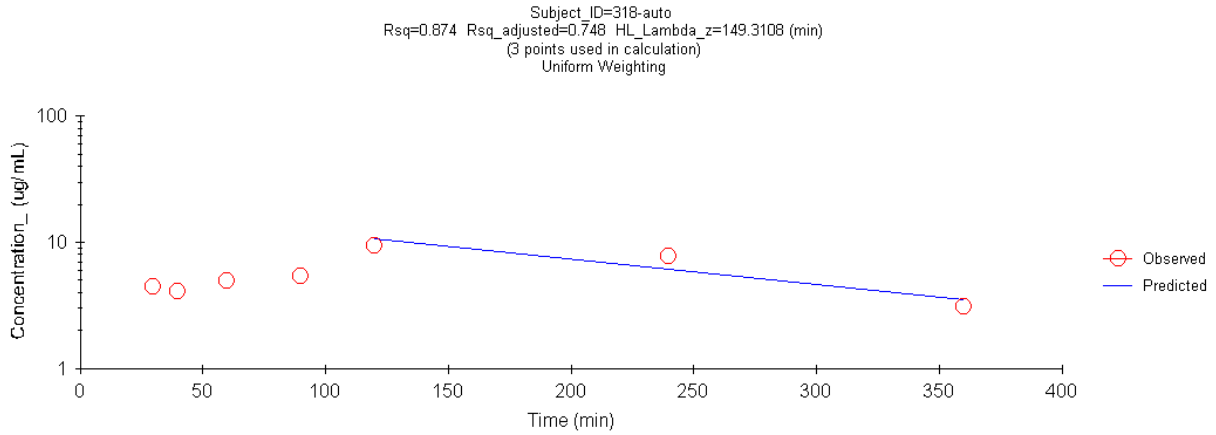
X vs. Observed Y and Predicted Y

C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



X vs. Observed Y and Predicted Y

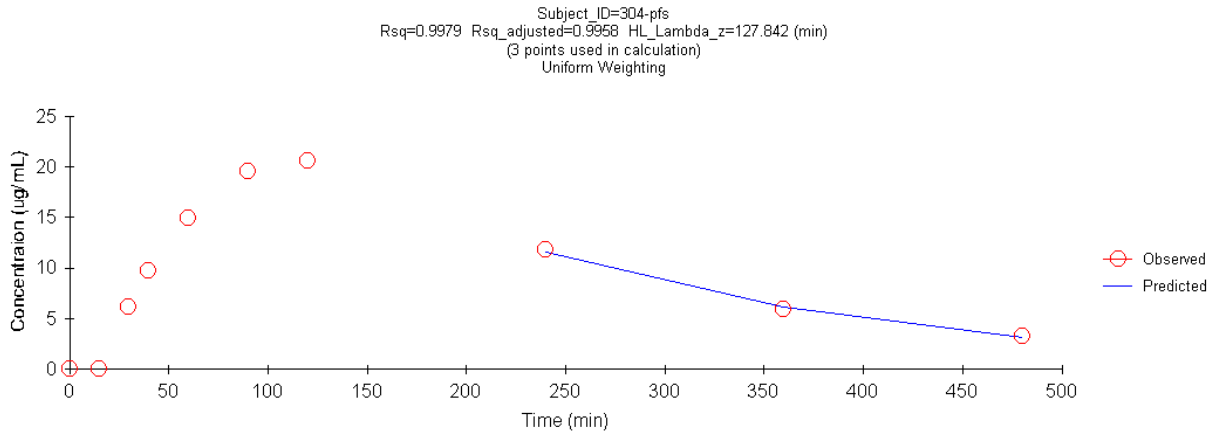
C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)



X vs. Observed Y and Predicted Y

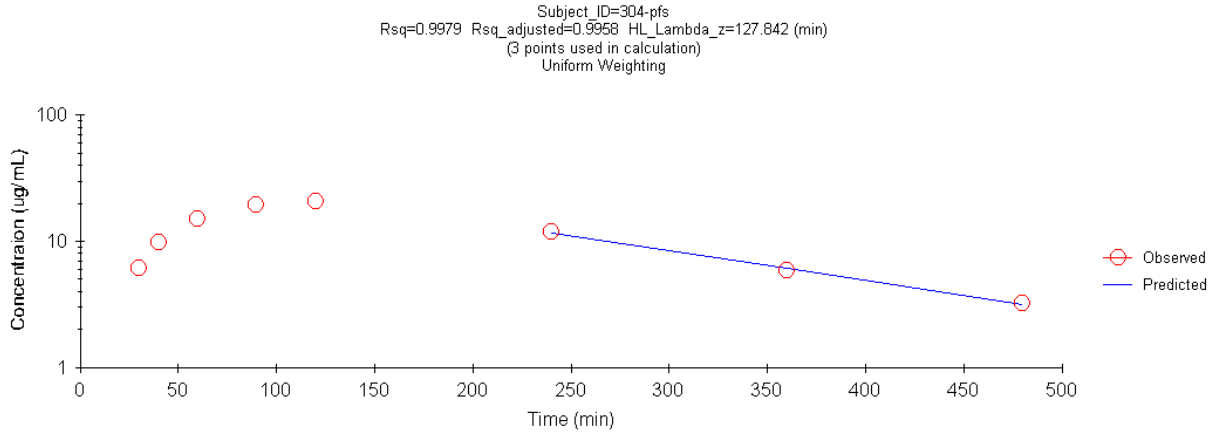
C:\Eric Edwards\Autoinj plots.pco (07-Oct-2011)

Treatment Group: Pre-Filled Syringe



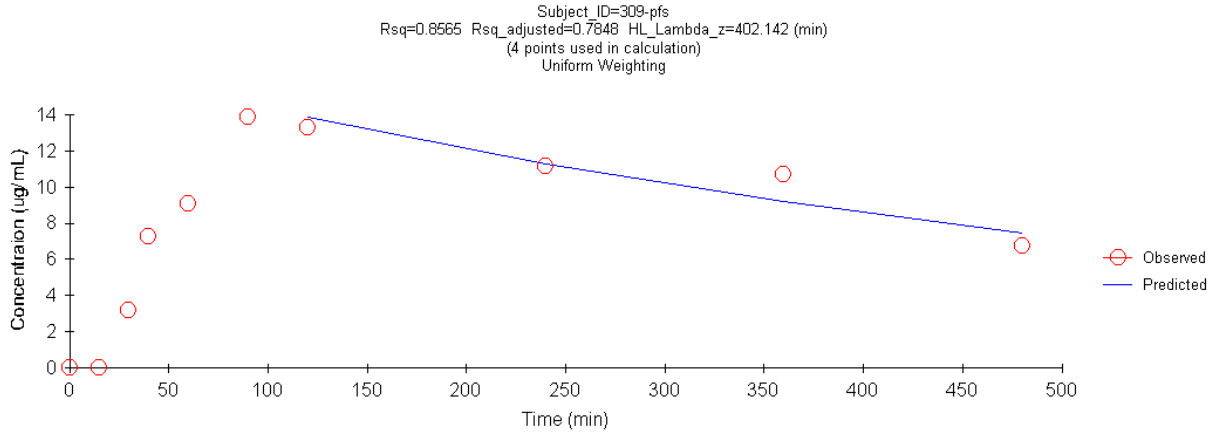
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



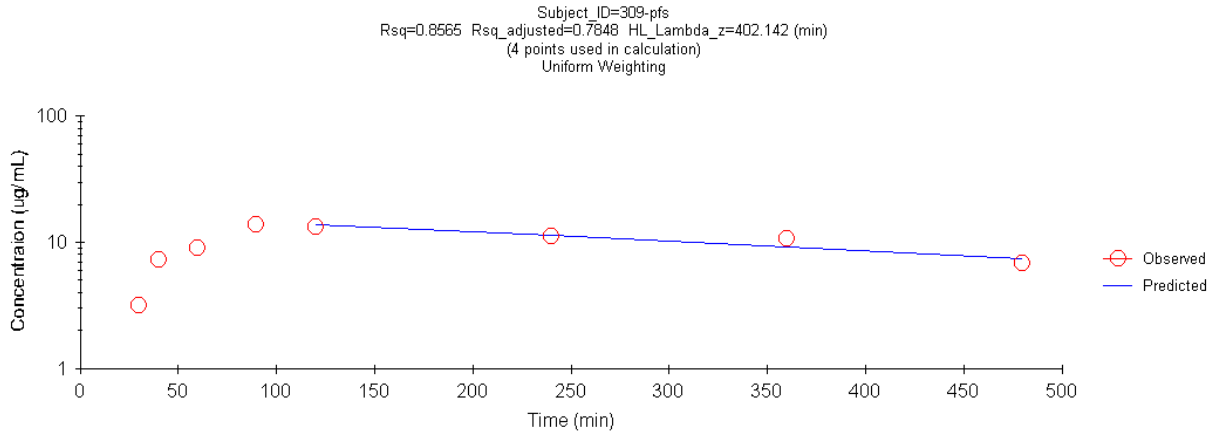
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



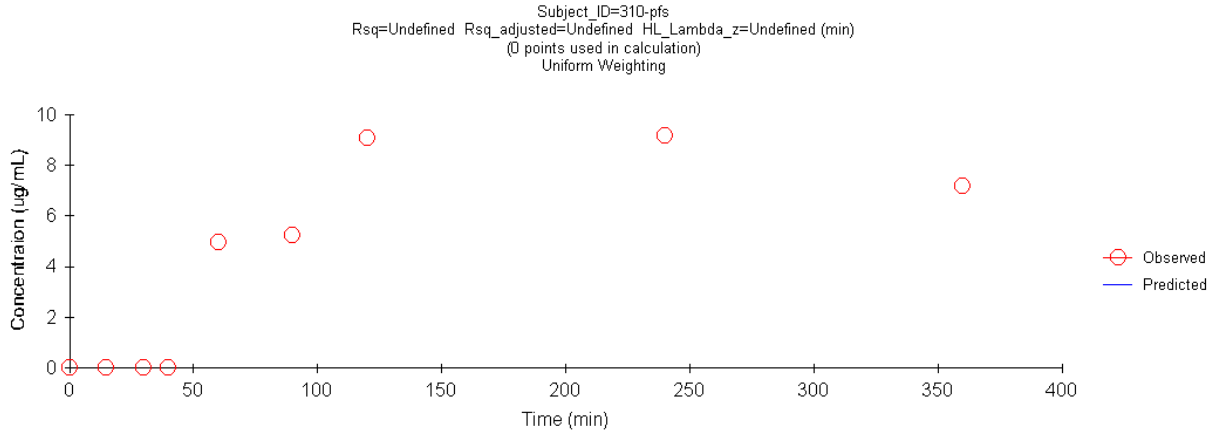
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



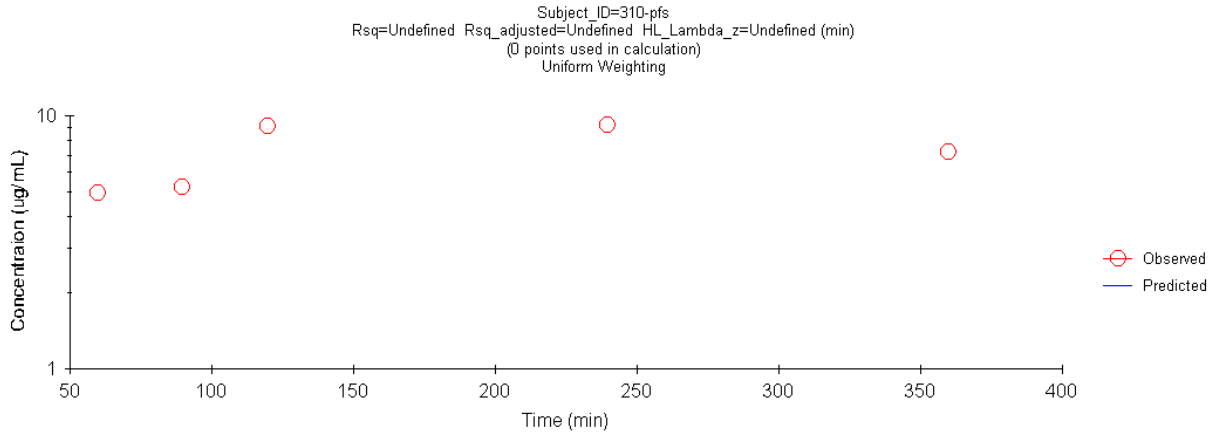
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



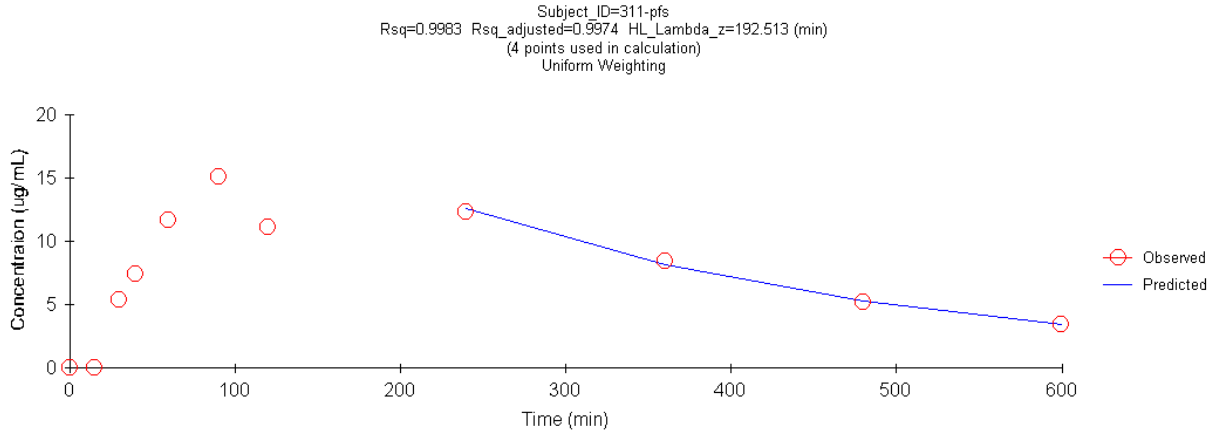
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



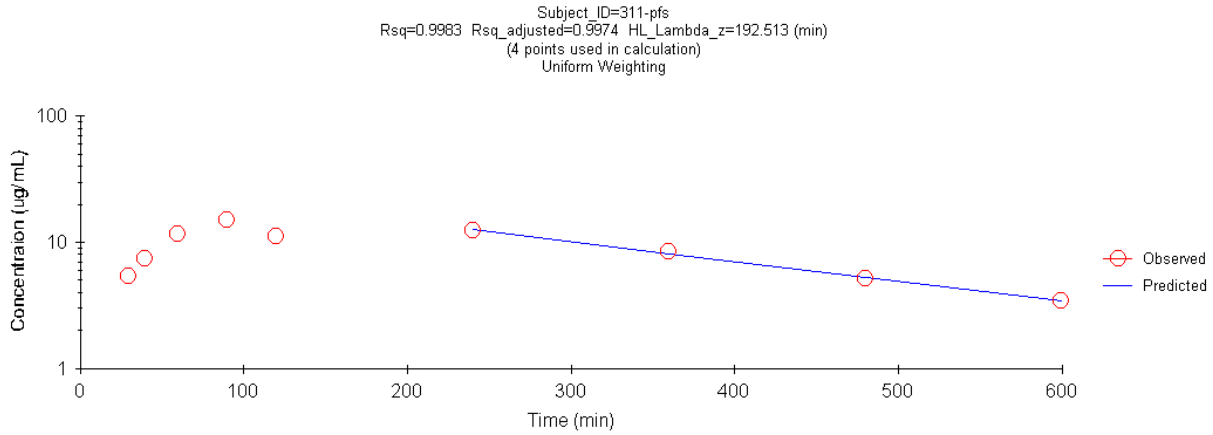
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



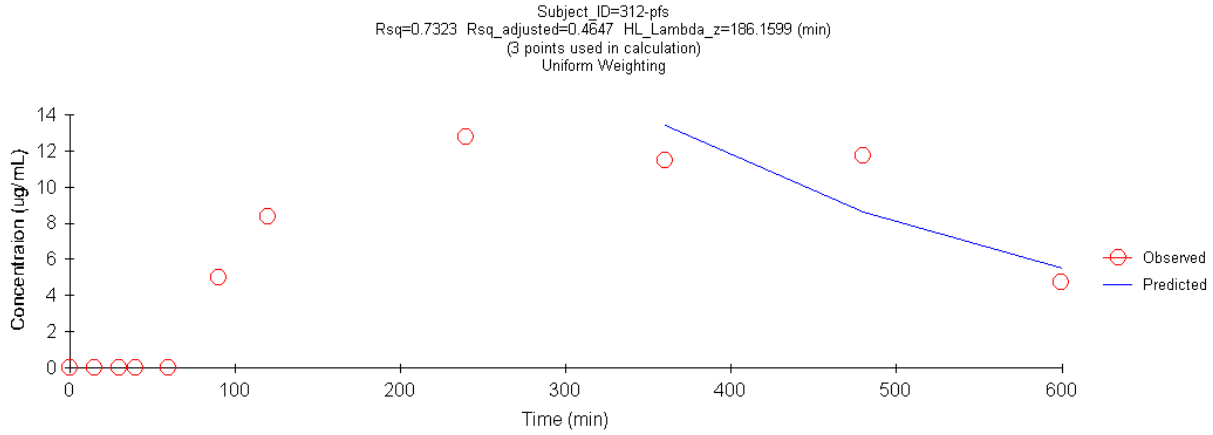
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



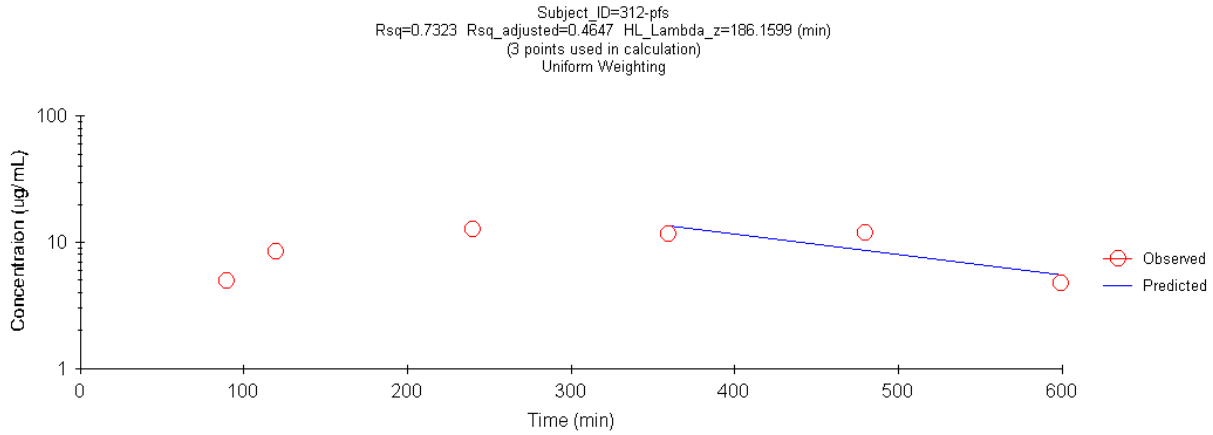
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



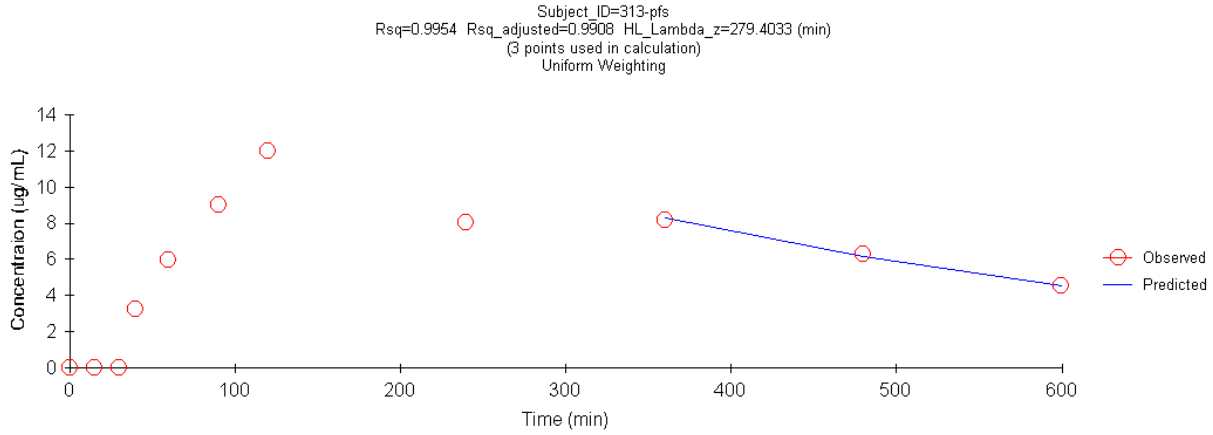
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



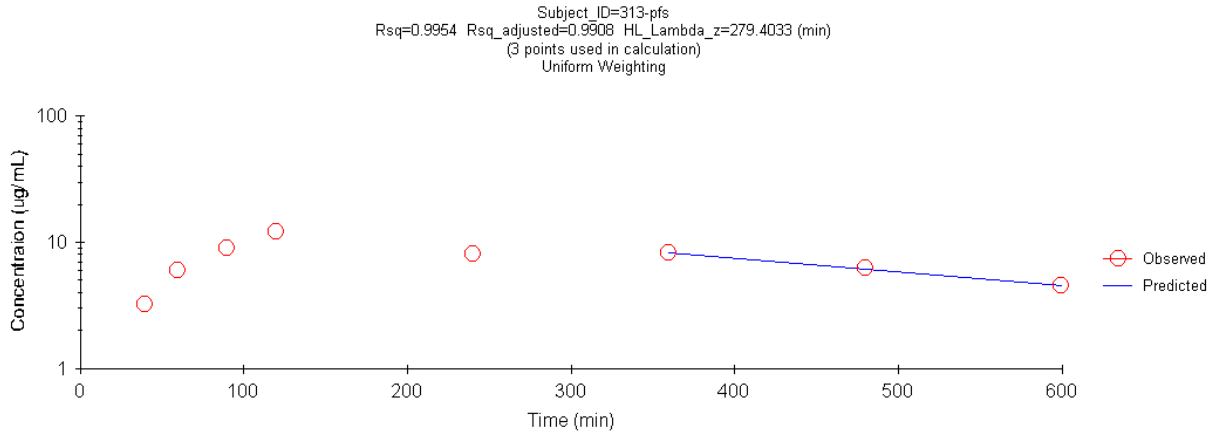
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



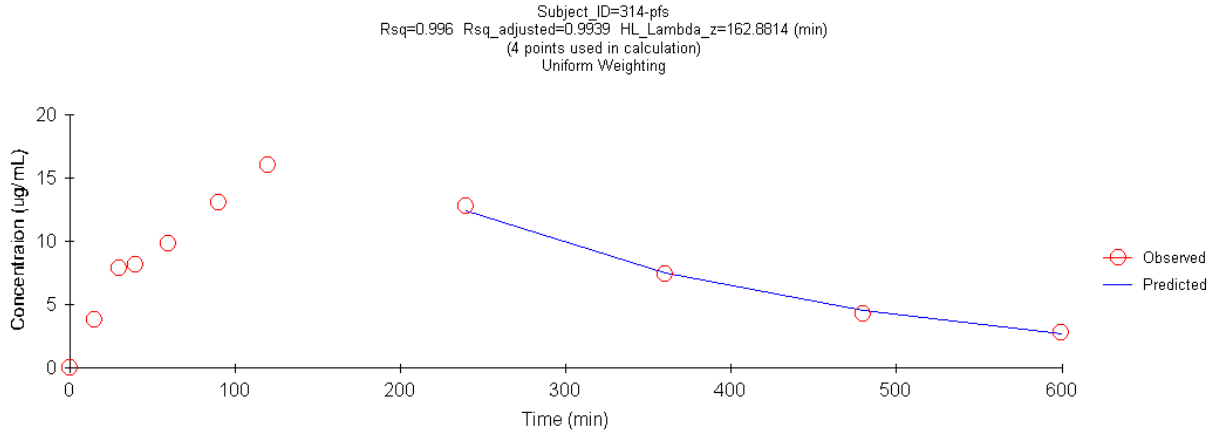
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



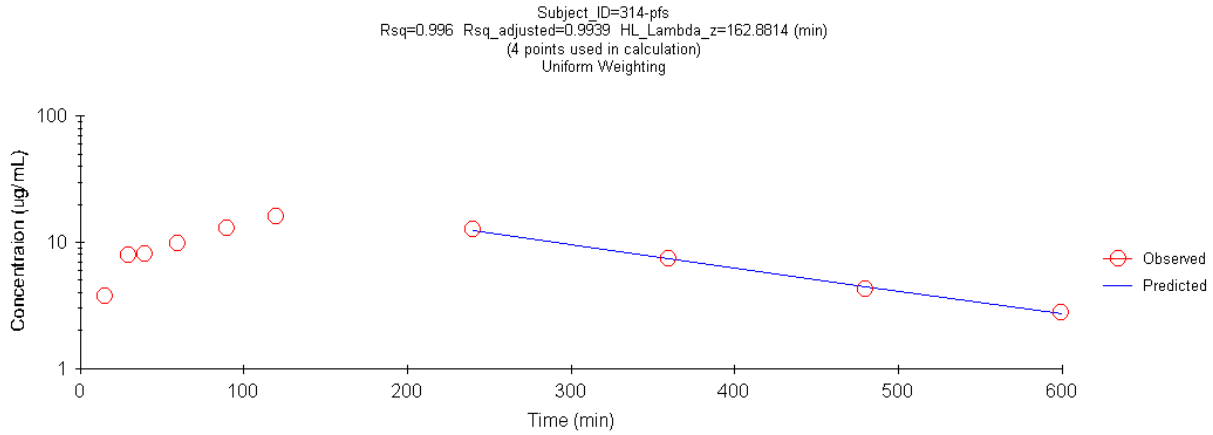
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



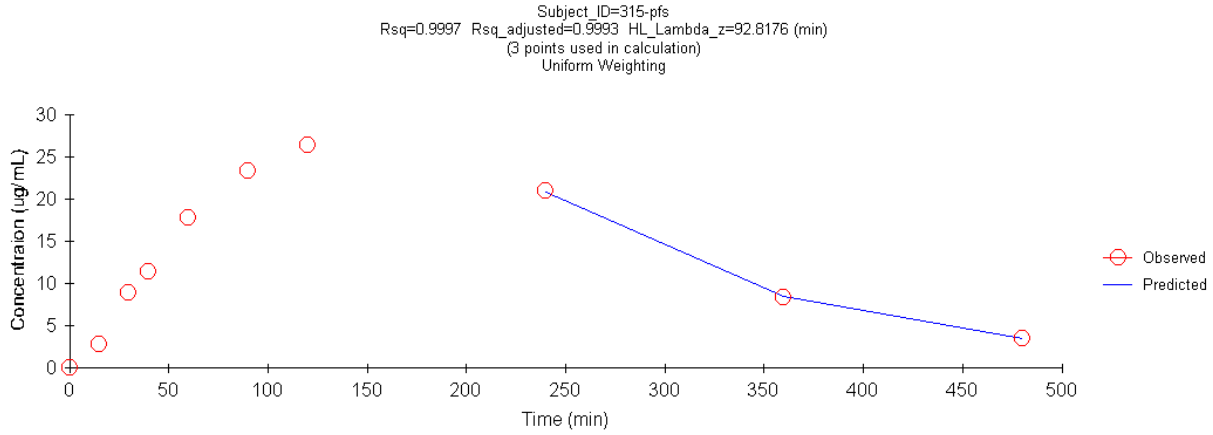
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



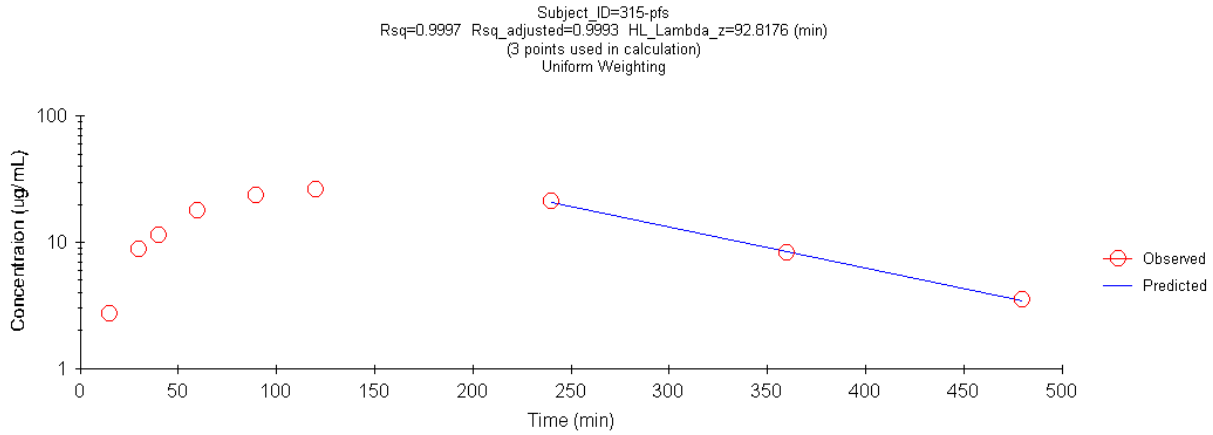
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



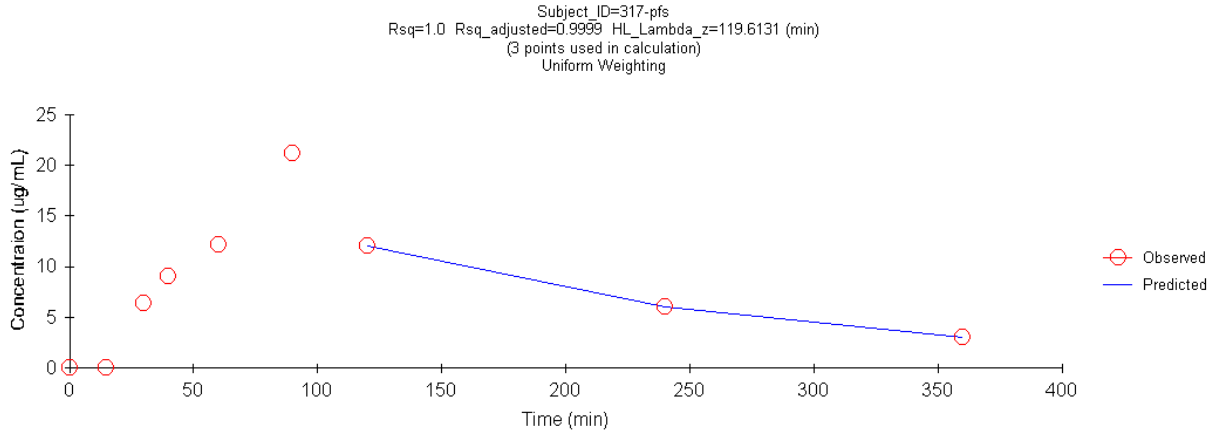
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



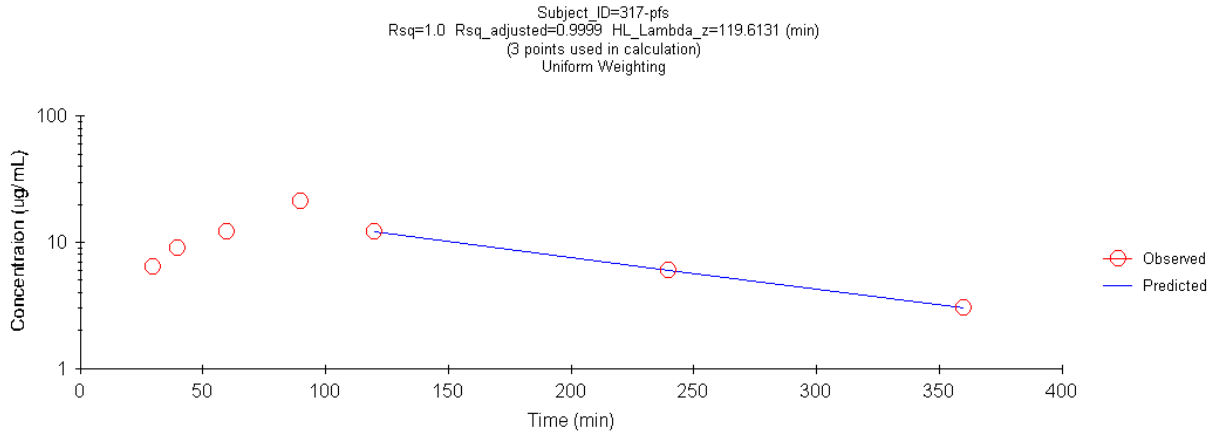
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



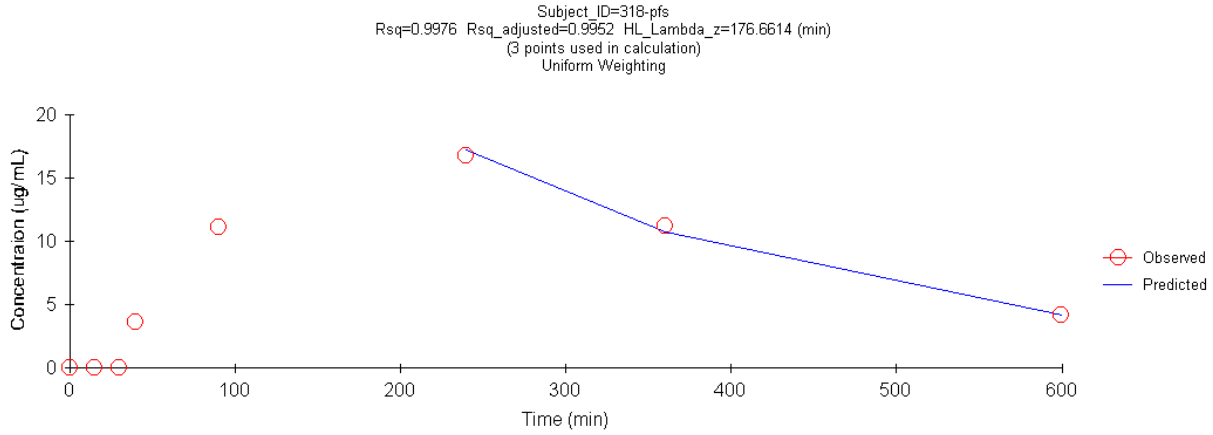
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



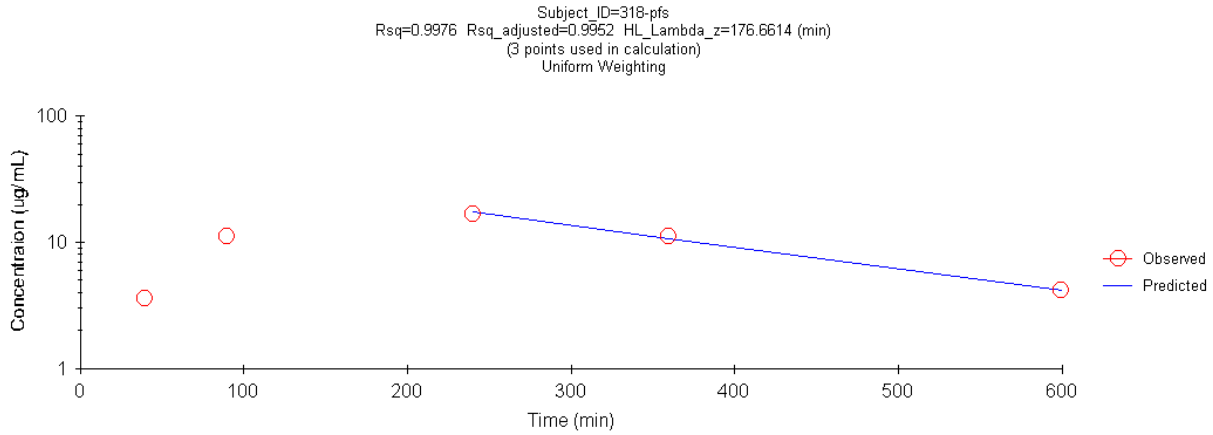
X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)



X vs. Observed Y and Predicted Y

C:\Eric Edwards\PFS plots.pco (07-Oct-2011)

APPENDIX K

Non-Compartmental Analyses Output Files for the Auto-Injector and Pre-filled Syringe Treatment Groups by Subject

Auto-Injector Results

```
Input File: Workbook - [C:\Er...\Data HM13424.xls]
Subject_ID=304-auto
                                                    Date:   10/06/2011
                                                    Time:     12:15:34

                    WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
                          Version 5.1  Build 200607251915
                              Core Version 18Apr2006
Settings
--------
Model:  Plasma Data, Extravascular Administration
Number of nonmissing observations:   9
Dose time:      0.00
Dose amount:      300.00
Calculation method:  Linear Trapezoidal Rule for Increasing Values,
                        Log Trapezoidal Rule for Decreasing Values
Weighting for lambda_z calculations:  Uniform weighting
Lambda_z method:  Find best fit for lambda_z,  Log regression
```

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		0.0000			0.0000	0.0000	
30.00		5.677			42.58	1277.	
40.00		9.100			116.5	3949.	
60.00		11.74			324.9	1.463e+004	
90.00		15.35			731.3	4.593e+004	
120.0	*	15.54	14.85	0.6829	1195.	9.462e+004	1.000
240.0	*	6.333	6.929	-0.5958	2425.	3.052e+005	1.000
360.0	*	3.381	3.232	0.1486	2990.	4.710e+005	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9897
Rsq_adjusted		0.9794
Corr_XY		-0.9948
No_points_lambda_z		3
Lambda_z	1/min	0.0064
Lambda_z_lower	min	120.0000
Lambda_z_upper	min	360.0000
HL_Lambda_z	min	109.0909
Tlag	min	15.0000
Tmax	min	120.0000
Cmax	ug/mL	15.5350

Cmax_D	ug/mL/mg	0.0518
Tlast	min	360.0000
Clast	ug/mL	3.3810
AUClast	min*ug/mL	2989.6819
AUCall	min*ug/mL	2989.6819
AUCINF_obs	min*ug/mL	3521.8002
AUCINF_D_obs	min*ug/mL/mg	11.7393
AUC_%Extrap_obs	%	15.1093
Vz_F_obs	mL	13406.6285
Cl_F_obs	mL/min	85.1837
AUCINF_pred	min*ug/mL	3498.4095
AUCINF_D_pred	min*ug/mL/mg	11.6614
AUC_%Extrap_pred	%	14.5417
Vz_F_pred	mL	13496.2665
Cl_F_pred	mL/min	85.7533
AUMClast	min*min*ug/mL	471040.4496
AUMCINF_obs	min*min*ug/mL	746350.4260
AUMC_%Extrap_obs	%	36.8875
AUMCINF_pred	min*min*ug/mL	734248.4225
AUMC_%Extrap_pred	%	35.8473
MRTlast	min	157.5554
MRTINF_obs	min	211.9230
MRTINF_pred	min	209.8806
AUC0_30	min*ug/mL	42.5775
AUC0_40	min*ug/mL	116.4625
AUC0_60	min*ug/mL	324.8925

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=309-auto

Date: 10/06/2011

Time: 12:15:34

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 10

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,

Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	3.180			23.85	357.8	
30.00	7.600			104.7	2426.	
40.00	10.64			195.9	5694.	
60.00	14.72			449.5	1.878e+004	

90.00	15.70			905.8	5.322e+004	
120.0	15.49			1374.	1.023e+005	
240.0 *	12.77	13.38	-0.6132	3064.	4.033e+005	1.000
360.0 *	8.440	7.684	0.7557	4319.	7.746e+005	1.000
480.0 *	4.210	4.412	-0.2022	5049.	1.076e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9790
Rsq_adjusted		0.9580
Corr_XY		-0.9895
No_points_lambda_z		3
Lambda_z	1/min	0.0046
Lambda_z_lower	min	240.0000
Lambda_z_upper	min	480.0000
HL_Lambda_z	min	149.9188
Tlag	min	0.0000
Tmax	min	90.0000
Cmax	ug/mL	15.7000
Cmax_D	ug/mL/mg	0.0523
Tlast	min	480.0000
Clast	ug/mL	4.2100
AUClast	min*ug/mL	5048.5278
AUCall	min*ug/mL	5048.5278
AUCINF_obs	min*ug/mL	5959.0967
AUCINF_D_obs	min*ug/mL/mg	19.8637
AUC_%Extrap_obs	%	15.2803
Vz_F_obs	mL	10888.5869

Cl_F_obs	mL/min	50.3432
AUCINF_pred	min*ug/mL	6002.8202
AUCINF_D_pred	min*ug/mL/mg	20.0094
AUC_%Extrap_pred	%	15.8974
Vz_F_pred	mL	10809.2763
Cl_F_pred	mL/min	49.9765
AUMClast	min*min*ug/mL	1076054.5534
AUMCINF_obs	min*min*ug/mL	1710071.9343
AUMC_%Extrap_obs	%	37.0755
AUMCINF_pred	min*min*ug/mL	1740516.0405
AUMC_%Extrap_pred	%	38.1761
MRTlast	min	213.1422
MRTINF_obs	min	286.9683
MRTINF_pred	min	289.9497
AUC0_30	min*ug/mL	104.7000
AUC0_40	min*ug/mL	195.9000
AUC0_60	min*ug/mL	449.5000

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=310-auto

Date: 10/06/2011

Time: 12:15:35

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 11

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,

Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 360.00, 600.00

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		0.0000			0.0000	0.0000	
30.00		4.240			31.80	954.0	
40.00		5.170			78.85	2624.	
60.00		8.710			217.7	9918.	
90.00		12.02			528.6	3.398e+004	
120.0		16.20			951.9	7.937e+004	
240.0		13.09			2703.	3.908e+005	
360.0	*	7.220	7.429	-0.2092	3887.	7.389e+005	1.000
480.0	*	6.500	6.139	0.3610	4709.	1.084e+006	1.000
600.0	*	4.930	5.073	-0.1429	5390.	1.450e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9370
Rsq_adjusted		0.8739
Corr_XY		-0.9680
No_points_lambda_z		3
Lambda_z	1/min	0.0016
Lambda_z_lower	min	360.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	436.0376
Tlag	min	15.0000
Tmax	min	120.0000
Cmax	ug/mL	16.2000
Cmax_D	ug/mL/mg	0.0540
Tlast	min	600.0000
Clast	ug/mL	4.9300
AUClast	min*ug/mL	5390.4627
AUCall	min*ug/mL	5390.4627
AUCINF_obs	min*ug/mL	8491.7746
AUCINF_D_obs	min*ug/mL/mg	28.3059
AUC_%Extrap_obs	%	36.5214
Vz_F_obs	mL	22223.9537
Cl_F_obs	mL/min	35.3283
AUCINF_pred	min*ug/mL	8581.6518
AUCINF_D_pred	min*ug/mL/mg	28.6055
AUC_%Extrap_pred	%	37.1862
Vz_F_pred	mL	21991.1981
Cl_F_pred	mL/min	34.9583
AUMClast	min*min*ug/mL	1449614.1824

AUMCINF_obs	min*min*ug/mL	5261341.5774
AUMC_%Extrap_obs	%	72.4478
AUMCINF_pred	min*min*ug/mL	5371806.9349
AUMC_%Extrap_pred	%	73.0144
MRTlast	min	268.9220
MRTINF_obs	min	619.5809
MRTINF_pred	min	625.9642
AUC0_30	min*ug/mL	31.8000
AUC0_40	min*ug/mL	78.8500
AUC0_60	min*ug/mL	217.6500

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=311-auto

Date: 10/06/2011

Time: 12:15:35

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 10

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,

Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 240.00,   480.00

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		0.0000			0.0000	0.0000	
30.00		5.983			44.87	1346.	
40.00		7.935			114.5	3831.	
60.00		14.74			341.2	1.585e+004	
90.00		16.40			808.3	5.125e+004	
120.0		16.60			1303.	1.033e+005	
240.0	*	12.27	11.34	0.9303	3022.	4.076e+005	1.000
360.0	*	5.341	6.253	-0.9121	4022.	6.993e+005	1.000
480.0	*	3.730	3.447	0.2827	4561.	9.235e+005	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9501
Rsq_adjusted		0.9001
Corr_XY		-0.9747
No_points_lambda_z		3
Lambda_z	1/min	0.0050
Lambda_z_lower	min	240.0000

Lambda_z_upper	min	480.0000
HL_Lambda_z	min	139.6778
Tlag	min	15.0000
Tmax	min	120.0000
Cmax	ug/mL	16.5980
Cmax_D	ug/mL/mg	0.0553
Tlast	min	480.0000
Clast	ug/mL	3.7300
AUClast	min*ug/mL	4560.8009
AUCall	min*ug/mL	4560.8009
AUCINF_obs	min*ug/mL	5312.4423
AUCINF_D_obs	min*ug/mL/mg	17.7081
AUC_%Extrap_obs	%	14.1487
Vz_F_obs	mL	11379.6494
Cl_F_obs	mL/min	56.4712
AUCINF_pred	min*ug/mL	5255.4651
AUCINF_D_pred	min*ug/mL/mg	17.5182
AUC_%Extrap_pred	%	13.2179
Vz_F_pred	mL	11503.0219
Cl_F_pred	mL/min	57.0834
AUMClast	min*min*ug/mL	923508.6062
AUMCINF_obs	min*min*ug/mL	1435761.5577
AUMC_%Extrap_obs	%	35.6781
AUMCINF_pred	min*min*ug/mL	1396930.9317
AUMC_%Extrap_pred	%	33.8902
MRTlast	min	202.4883
MRTINF_obs	min	270.2639
MRTINF_pred	min	265.8054
AUC0_30	min*ug/mL	44.8725

AUC0_40 min*ug/mL 114.4625

AUC0_60 min*ug/mL 341.1825

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=312-auto

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WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 11

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,

Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	

15.00		4.240			31.80	477.0	
30.00		5.440			104.4	2178.	
40.00		7.400			168.6	4474.	
60.00		14.42			386.8	1.609e+004	
90.00		16.10			844.6	5.080e+004	
120.0		17.72			1352.	1.044e+005	
240.0		17.23			3449.	4.813e+005	
360.0	*	12.03	12.08	-0.04574	5186.	9.961e+005	1.000
480.0	*	7.060	7.007	0.05338	6305.	1.460e+006	1.000
600.0	*	4.050	4.065	-0.01540	6955.	1.808e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9999
Rsq_adjusted		0.9997
Corr_XY		-0.9999
No_points_lambda_z		3
Lambda_z	1/min	0.0045
Lambda_z_lower	min	360.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	152.8037
Tlag	min	0.0000
Tmax	min	120.0000
Cmax	ug/mL	17.7200
Cmax_D	ug/mL/mg	0.0591
Tlast	min	600.0000
Clast	ug/mL	4.0500
AUClast	min*ug/mL	6954.7163

AUCall	min*ug/mL	6954.7163
AUCINF_obs	min*ug/mL	7847.5352
AUCINF_D_obs	min*ug/mL/mg	26.1585
AUC_%Extrap_obs	%	11.3771
Vz_F_obs	mL	8427.4528
Cl_F_obs	mL/min	38.2286
AUCINF_pred	min*ug/mL	7850.9298
AUCINF_D_pred	min*ug/mL/mg	26.1698
AUC_%Extrap_pred	%	11.4154
Vz_F_pred	mL	8423.8089
Cl_F_pred	mL/min	38.2120
AUMClast	min*min*ug/mL	1807581.5842
AUMCINF_obs	min*min*ug/mL	2540094.0415
AUMC_%Extrap_obs	%	28.8380
AUMCINF_pred	min*min*ug/mL	2542879.1700
AUMC_%Extrap_pred	%	28.9159
MRTlast	min	259.9073
MRTINF_obs	min	323.6805
MRTINF_pred	min	323.8953
AUC0_30	min*ug/mL	104.4000
AUC0_40	min*ug/mL	168.6000
AUC0_60	min*ug/mL	386.8000

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Subject_ID=313-auto

Date: 10/06/2011

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WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 10

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,

Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 500.00, 504.00

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	0.0000			0.0000	0.0000	
30.00	0.0000			0.0000	0.0000	
40.00	0.0000			0.0000	0.0000	
60.00	0.0000			0.0000	0.0000	
90.00	3.811			57.17	5145.	
120.0	6.312			209.0	2.165e+004	
240.0	7.267			1024.	1.717e+005	
360.0	3.727			1660.	3.584e+005	

480.0 4.976 2182. 5.822e+005

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14530: Lambda_z could not be estimated.

No parameters could be extrapolated to infinity.

Final Parameters

Rsq		Missing
Rsq_adjusted		Missing
Corr_XY		Missing
No_points_lambda_z		0
Lambda_z	1/min	Missing
Lambda_z_lower	min	Missing
Lambda_z_upper	min	Missing
HL_Lambda_z	min	Missing
Tlag	min	60.0000
Tmax	min	240.0000
Cmax	ug/mL	7.2670
Cmax_D	ug/mL/mg	0.0242
Tlast	min	480.0000
Clast	ug/mL	4.9760
AUClast	min*ug/mL	2182.1058
AUCall	min*ug/mL	2182.1058
AUCINF_obs	min*ug/mL	Missing
AUCINF_D_obs	min*ug/mL/mg	Missing
AUC_%Extrap_obs	%	Missing
Vz_F_obs	mL	Missing
Cl_F_obs	mL/min	Missing
AUCINF_pred	min*ug/mL	Missing

AUCINF_D_pred	min*ug/mL/mg	Missing
AUC_%Extrap_pred	%	Missing
Vz_F_pred	mL	Missing
Cl_F_pred	mL/min	Missing
AUMClast	min*min*ug/mL	582190.4834
AUMCINF_obs	min*min*ug/mL	Missing
AUMC_%Extrap_obs	%	Missing
AUMCINF_pred	min*min*ug/mL	Missing
AUMC_%Extrap_pred	%	Missing
MRTlast	min	266.8021
MRTINF_obs	min	Missing
MRTINF_pred	min	Missing
AUC0_30	min*ug/mL	0.0000
AUC0_40	min*ug/mL	0.0000
AUC0_60	min*ug/mL	0.0000

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=314-auto

Date: 10/06/2011

Time: 12:15:35

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 8

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,
Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

	Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
	min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
	0.0000	0.0000			0.0000	0.0000	
	15.00	0.0000			0.0000	0.0000	
	30.00	4.098			30.74	922.1	
	40.00	5.413			78.29	2619.	
	60.00	10.92			241.6	1.134e+004	
	90.00 *	15.05	16.11	-1.062	631.1	4.148e+004	1.000
	120.0 *	13.86	12.72	1.133	1064.	8.688e+004	1.000
	240.0 *	4.871	4.955	-0.08381	2096.	2.619e+005	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq	0.9845
Rsq_adjusted	0.9691
Corr_XY	-0.9922
No_points_lambda_z	3

Lambda_z	1/min	0.0079
Lambda_z_lower	min	90.0000
Lambda_z_upper	min	240.0000
HL_Lambda_z	min	88.1924
Tlag	min	15.0000
Tmax	min	90.0000
Cmax	ug/mL	15.0450
Cmax_D	ug/mL/mg	0.0502
Tlast	min	240.0000
Clast	ug/mL	4.8710
AUClast	min*ug/mL	2095.7810
AUCall	min*ug/mL	2095.7810
AUCINF_obs	min*ug/mL	2715.5416
AUCINF_D_obs	min*ug/mL/mg	9.0518
AUC_%Extrap_obs	%	22.8227
Vz_F_obs	mL	14056.2876
Cl_F_obs	mL/min	110.4752
AUCINF_pred	min*ug/mL	2726.2047
AUCINF_D_pred	min*ug/mL/mg	9.0873
AUC_%Extrap_pred	%	23.1246
Vz_F_pred	mL	14001.3089
Cl_F_pred	mL/min	110.0431
AUMClast	min*min*ug/mL	261941.5317
AUMCINF_obs	min*min*ug/mL	489539.1875
AUMC_%Extrap_obs	%	46.4922
AUMCINF_pred	min*min*ug/mL	493455.0403
AUMC_%Extrap_pred	%	46.9168
MRTlast	min	124.9852
MRTINF_obs	min	180.2731

MRTINF_pred	min	181.0044
AUC0_30	min*ug/mL	30.7350
AUC0_40	min*ug/mL	78.2900
AUC0_60	min*ug/mL	241.6200

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=315-auto

Date: 10/06/2011

Time: 12:15:35

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 11

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,

Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	

0.0000		0.0000			0.0000	0.0000	
15.00		7.830			58.73	880.9	
30.00		14.53			226.4	5031.	
40.00		19.29			395.5	1.107e+004	
60.00		27.74			865.8	3.543e+004	
90.00	*	29.80	32.27	-2.475	1729.	1.006e+005	1.000
120.0	*	28.73	27.88	0.8540	2607.	1.927e+005	1.000
240.0	*	17.48	15.51	1.967	5324.	6.683e+005	1.000
360.0	*	8.520	8.633	-0.1134	6820.	1.107e+006	1.000
480.0	*	4.290	4.805	-0.5146	7560.	1.412e+006	1.000
600.0	*	2.830	2.674	0.1562	7981.	1.638e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9923
Rsq_adjusted		0.9904
Corr_XY		-0.9962
No_points_lambda_z		6
Lambda_z	1/min	0.0049
Lambda_z_lower	min	90.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	141.9265
Tlag	min	0.0000
Tmax	min	90.0000
Cmax	ug/mL	29.8000
Cmax_D	ug/mL/mg	0.0993
Tlast	min	600.0000

Clast	ug/mL	2.8300
AUClast	min*ug/mL	7980.8092
AUCall	min*ug/mL	7980.8092
AUCINF_obs	min*ug/mL	8560.2706
AUCINF_D_obs	min*ug/mL/mg	28.5342
AUC_%Extrap_obs	%	6.7692
Vz_F_obs	mL	7175.8245
Cl_F_obs	mL/min	35.0456
AUCINF_pred	min*ug/mL	8528.2974
AUCINF_D_pred	min*ug/mL/mg	28.4277
AUC_%Extrap_pred	%	6.4197
Vz_F_pred	mL	7202.7272
Cl_F_pred	mL/min	35.1770
AUMClast	min*min*ug/mL	1637858.6360
AUMCINF_obs	min*min*ug/mL	2104184.0302
AUMC_%Extrap_obs	%	22.1618
AUMCINF_pred	min*min*ug/mL	2078453.3510
AUMC_%Extrap_pred	%	21.1982
MRTlast	min	205.2246
MRTINF_obs	min	245.8081
MRTINF_pred	min	243.7126
AUC0_30	min*ug/mL	226.4250
AUC0_40	min*ug/mL	395.5250
AUC0_60	min*ug/mL	865.8250

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=317-auto

Date: 10/06/2011

Time: 12:15:36

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 10

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,

Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 120.00, 480.00

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	5.830			43.73	655.9	
30.00	13.85			191.3	4428.	
40.00	15.99			340.5	9704.	
60.00	22.57			726.1	2.964e+004	
90.00	27.03			1470.	8.645e+004	
120.0 *	24.92	24.54	0.3799	2249.	1.681e+005	1.000

240.0 *	12.29	12.97	-0.6797	4393.	5.390e+005	1.000
360.0 *	7.290	6.855	0.4354	5542.	8.776e+005	1.000
480.0 *	3.540	3.623	-0.08275	6165.	1.135e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9963
Rsq_adjusted		0.9945
Corr_XY		-0.9982
No_points_lambda_z		4
Lambda_z	1/min	0.0053
Lambda_z_lower	min	120.0000
Lambda_z_upper	min	480.0000
HL_Lambda_z	min	130.4356
Tlag	min	0.0000
Tmax	min	90.0000
Cmax	ug/mL	27.0300
Cmax_D	ug/mL/mg	0.0901
Tlast	min	480.0000
Clast	ug/mL	3.5400
AUClast	min*ug/mL	6164.7490
AUCall	min*ug/mL	6164.7490
AUCINF_obs	min*ug/mL	6830.9019
AUCINF_D_obs	min*ug/mL/mg	22.7697
AUC_%Extrap_obs	%	9.7520
Vz_F_obs	mL	8264.4484
Cl_F_obs	mL/min	43.9181
AUCINF_pred	min*ug/mL	6846.4745

AUCINF_D_pred	min*ug/mL/mg	22.8216
AUC_%Extrap_pred	%	9.9573
Vz_F_pred	mL	8245.6506
Cl_F_pred	mL/min	43.8182
AUMClast	min*min*ug/mL	1134804.9002
AUMCINF_obs	min*min*ug/mL	1579914.1439
AUMC_%Extrap_obs	%	28.1730
AUMCINF_pred	min*min*ug/mL	1590319.4221
AUMC_%Extrap_pred	%	28.6430
MRTlast	min	184.0797
MRTINF_obs	min	231.2892
MRTINF_pred	min	232.2830
AUC0_30	min*ug/mL	191.3250
AUC0_40	min*ug/mL	340.5250
AUC0_60	min*ug/mL	726.1250

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=318-auto

Date: 10/06/2011

Time: 12:15:36

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 9

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal Rule for Increasing Values,
Log Trapezoidal Rule for Decreasing Values

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		0.0000			0.0000	0.0000	
30.00		4.402			33.02	990.5	
40.00		4.116			75.59	2478.	
60.00		4.909			165.8	7070.	
90.00		5.344			319.6	1.870e+004	
120.0	*	9.467	10.70	-1.230	541.8	4.296e+004	1.000
240.0	*	7.823	6.128	1.695	1576.	2.272e+005	1.000
360.0	*	3.107	3.511	-0.4035	2189.	4.054e+005	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq	0.8740
Rsq_adjusted	0.7480
Corr_XY	-0.9349
No_points_lambda_z	3

Lambda_z	1/min	0.0046
Lambda_z_lower	min	120.0000
Lambda_z_upper	min	360.0000
HL_Lambda_z	min	149.3108
Tlag	min	15.0000
Tmax	min	120.0000
Cmax	ug/mL	9.4670
Cmax_D	ug/mL/mg	0.0316
Tlast	min	360.0000
Clast	ug/mL	3.1070
AUClast	min*ug/mL	2188.9236
AUCall	min*ug/mL	2188.9236
AUCINF_obs	min*ug/mL	2858.2024
AUCINF_D_obs	min*ug/mL/mg	9.5273
AUC_%Extrap_obs	%	23.4161
Vz_F_obs	mL	22609.6644
Cl_F_obs	mL/min	104.9611
AUCINF_pred	min*ug/mL	2945.1281
AUCINF_D_pred	min*ug/mL/mg	9.8171
AUC_%Extrap_pred	%	25.6765
Vz_F_pred	mL	21942.3384
Cl_F_pred	mL/min	101.8631
AUMClast	min*min*ug/mL	405430.9329
AUMCINF_obs	min*min*ug/mL	790540.6716
AUMC_%Extrap_obs	%	48.7147
AUMCINF_pred	min*min*ug/mL	840558.5746
AUMC_%Extrap_pred	%	51.7665
MRTlast	min	185.2193
MRTINF_obs	min	276.5867

MRTINF_pred	min	285.4065
AUC0_30	min*ug/mL	33.0150
AUC0_40	min*ug/mL	75.5890
AUC0_60	min*ug/mL	165.8390

Pre-filled Syringe Results

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=304-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 10

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 240.00, 480.00

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		0.0000			0.0000	0.0000	
30.00		6.150			46.13	1384.	
40.00		9.750			125.6	4256.	
60.00		14.94			372.5	1.712e+004	
90.00		19.53			889.6	5.693e+004	
120.0		20.62			1492.	1.204e+005	
240.0	*	11.83	11.63	0.2027	3439.	4.392e+005	1.000
360.0	*	5.860	6.066	-0.2061	4500.	7.362e+005	1.000
480.0	*	3.220	3.165	0.05519	5045.	9.555e+005	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9979
Rsq_adjusted		0.9958
Corr_XY		-0.9989
No_points_lambda_z		3
Lambda_z	1/min	0.0054
Lambda_z_lower	min	240.0000
Lambda_z_upper	min	480.0000
HL_Lambda_z	min	127.8420
Tlag	min	15.0000
Tmax	min	120.0000
Cmax	ug/mL	20.6200
Cmax_D	ug/mL/mg	0.0687

Tlast	min	480.0000
Clast	ug/mL	3.2200
AUClast	min*ug/mL	5045.0250
AUCall	min*ug/mL	5045.0250
AUCINF_obs	min*ug/mL	5638.9121
AUCINF_D_obs	min*ug/mL/mg	18.7964
AUC_%Extrap_obs	%	10.5319
Vz_F_obs	mL	9812.3715
Cl_F_obs	mL/min	53.2018
AUCINF_pred	min*ug/mL	5628.7340
AUCINF_D_pred	min*ug/mL/mg	18.7624
AUC_%Extrap_pred	%	10.3702
Vz_F_pred	mL	9830.1148
Cl_F_pred	mL/min	53.2980
AUMClast	min*min*ug/mL	955469.2500
AUMCINF_obs	min*min*ug/mL	1350069.8480
AUMC_%Extrap_obs	%	29.2282
AUMCINF_pred	min*min*ug/mL	1343307.0860
AUMC_%Extrap_pred	%	28.8719
MRTlast	min	189.3884
MRTINF_obs	min	239.4203
MRTINF_pred	min	238.6517
AUC0_30	min*ug/mL	46.1250
AUC0_40	min*ug/mL	125.6250
AUC0_60	min*ug/mL	372.5250

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=309-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 10

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 120.00, 480.00

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	0.0000			0.0000	0.0000	
30.00	3.180			23.85	715.5	
40.00	7.257			76.04	2644.	
60.00	9.093			239.5	1.100e+004	

90.00	13.90			584.4	3.794e+004	
120.0 *	13.28	13.89	-0.6092	992.0	8.061e+004	1.000
240.0 *	11.18	11.29	-0.1110	2460.	3.373e+005	1.000
360.0 *	10.72	9.184	1.532	3774.	7.298e+005	1.000
480.0 *	6.760	7.468	-0.7078	4822.	1.156e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.8565
Rsq_adjusted		0.7848
Corr_XY		-0.9255
No_points_lambda_z		4
Lambda_z	1/min	0.0017
Lambda_z_lower	min	120.0000
Lambda_z_upper	min	480.0000
HL_Lambda_z	min	402.1420
Tlag	min	15.0000
Tmax	min	90.0000
Cmax	ug/mL	13.8950
Cmax_D	ug/mL/mg	0.0463
Tlast	min	480.0000
Clast	ug/mL	6.7600
AUClast	min*ug/mL	4822.2600
AUCall	min*ug/mL	4822.2600
AUCINF_obs	min*ug/mL	8744.1972
AUCINF_D_obs	min*ug/mL/mg	29.1473
AUC_%Extrap_obs	%	44.8519
Vz_F_obs	mL	19904.6825

Cl_F_obs	mL/min	34.3085
AUCINF_pred	min*ug/mL	9154.8334
AUCINF_D_pred	min*ug/mL/mg	30.5161
AUC_%Extrap_pred	%	47.3255
Vz_F_pred	mL	19011.8664
Cl_F_pred	mL/min	32.7696
AUMClast	min*min*ug/mL	1155912.3000
AUMCINF_obs	min*min*ug/mL	5313825.5747
AUMC_%Extrap_obs	%	78.2471
AUMCINF_pred	min*min*ug/mL	5749169.0011
AUMC_%Extrap_pred	%	79.8943
MRTlast	min	239.7034
MRTINF_obs	min	607.6974
MRTINF_pred	min	627.9927
AUC0_30	min*ug/mL	23.8500
AUC0_40	min*ug/mL	76.0350
AUC0_60	min*ug/mL	239.5350

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=310-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 9

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	0.0000			0.0000	0.0000	
30.00	0.0000			0.0000	0.0000	
40.00	0.0000			0.0000	0.0000	
60.00	4.934			49.34	2960.	
90.00	5.249			202.1	1.449e+004	
120.0	9.066			416.8	3.789e+004	
240.0	9.170			1511.	2.352e+005	
360.0	7.200			2493.	5.228e+005	

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14530: Lambda_z could not be estimated.

No parameters could be extrapolated to infinity.

Final Parameters

Rsq	Missing

Rsq_adjusted		Missing
Corr_XY		Missing
No_points_lambda_z		0
Lambda_z	1/min	Missing
Lambda_z_lower	min	Missing
Lambda_z_upper	min	Missing
HL_Lambda_z	min	Missing
Tlag	min	40.0000
Tmax	min	240.0000
Cmax	ug/mL	9.1700
Cmax_D	ug/mL/mg	0.0306
Tlast	min	360.0000
Clast	ug/mL	7.2000
AUClast	min*ug/mL	2493.1700
AUCall	min*ug/mL	2493.1700
AUCINF_obs	min*ug/mL	Missing
AUCINF_D_obs	min*ug/mL/mg	Missing
AUC_%Extrap_obs	%	Missing
Vz_F_obs	mL	Missing
Cl_F_obs	mL/min	Missing
AUCINF_pred	min*ug/mL	Missing
AUCINF_D_pred	min*ug/mL/mg	Missing
AUC_%Extrap_pred	%	Missing
Vz_F_pred	mL	Missing
Cl_F_pred	mL/min	Missing
AUMClast	min*min*ug/mL	522783.3000
AUMCINF_obs	min*min*ug/mL	Missing
AUMC_%Extrap_obs	%	Missing
AUMCINF_pred	min*min*ug/mL	Missing

AUMC_%Extrap_pred	%	Missing
MRTlast	min	209.6862
MRTINF_obs	min	Missing
MRTINF_pred	min	Missing
AUC0_30	min*ug/mL	0.0000
AUC0_40	min*ug/mL	0.0000
AUC0_60	min*ug/mL	49.3400

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=311-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 11

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		0.0000			0.0000	0.0000	
30.00		5.350			40.13	1204.	
40.00		7.400			103.9	3486.	
60.00		11.72			295.1	1.348e+004	
90.00		15.08			697.1	4.438e+004	
120.0		11.14			1090.	8.479e+004	
240.0	*	12.37	12.57	-0.2008	2501.	3.431e+005	1.000
360.0	*	8.430	8.161	0.2694	3749.	7.033e+005	1.000
480.0	*	5.210	5.298	-0.08758	4567.	1.035e+006	1.000
600.0	*	3.440	3.439	0.0009791	5086.	1.309e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9983
Rsq_adjusted		0.9974
Corr_XY		-0.9991
No_points_lambda_z		4
Lambda_z	1/min	0.0036
Lambda_z_lower	min	240.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	192.5130
Tlag	min	15.0000
Tmax	min	90.0000
Cmax	ug/mL	15.0800

Cmax_D	ug/mL/mg	0.0503
Tlast	min	600.0000
Clast	ug/mL	3.4400
AUClast	min*ug/mL	5086.3750
AUCall	min*ug/mL	5086.3750
AUCINF_obs	min*ug/mL	6041.7923
AUCINF_D_obs	min*ug/mL/mg	20.1393
AUC_%Extrap_obs	%	15.8135
Vz_F_obs	mL	13790.8212
Cl_F_obs	mL/min	49.6541
AUCINF_pred	min*ug/mL	6041.5204
AUCINF_D_pred	min*ug/mL/mg	20.1384
AUC_%Extrap_pred	%	15.8097
Vz_F_pred	mL	13791.4419
Cl_F_pred	mL/min	49.6564
AUMClast	min*min*ug/mL	1309370.2500
AUMCINF_obs	min*min*ug/mL	2147975.9443
AUMC_%Extrap_obs	%	39.0417
AUMCINF_pred	min*min*ug/mL	2147737.2615
AUMC_%Extrap_pred	%	39.0349
MRTlast	min	257.4270
MRTINF_obs	min	355.5197
MRTINF_pred	min	355.4962
AUC0_30	min*ug/mL	40.1250
AUC0_40	min*ug/mL	103.8750
AUC0_60	min*ug/mL	295.0750

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=312-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 11

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 360.00, 600.00

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	0.0000			0.0000	0.0000	
30.00	0.0000			0.0000	0.0000	
40.00	0.0000			0.0000	0.0000	
60.00	0.0000			0.0000	0.0000	

90.00		4.956			74.34	6691.	
120.0		8.370			274.2	2.845e+004	
240.0		12.78			1543.	2.728e+005	
360.0	*	11.51	13.45	-1.943	3001.	7.055e+005	1.000
480.0	*	11.76	8.606	3.150	4397.	1.293e+006	1.000
600.0	*	4.710	5.505	-0.7949	5385.	1.801e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.7323
Rsq_adjusted		0.4647
Corr_XY		-0.8558
No_points_lambda_z		3
Lambda_z	1/min	0.0037
Lambda_z_lower	min	360.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	186.1599
Tlag	min	60.0000
Tmax	min	240.0000
Cmax	ug/mL	12.7830
Cmax_D	ug/mL/mg	0.0426
Tlast	min	600.0000
Clast	ug/mL	4.7100
AUClast	min*ug/mL	5385.0300
AUCall	min*ug/mL	5385.0300
AUCINF_obs	min*ug/mL	6650.0040
AUCINF_D_obs	min*ug/mL/mg	22.1667
AUC_%Extrap_obs	%	19.0222

Vz_F_obs	mL	12116.0218
Cl_F_obs	mL/min	45.1128
AUCINF_pred	min*ug/mL	6863.4998
AUCINF_D_pred	min*ug/mL/mg	22.8783
AUC_%Extrap_pred	%	21.5410
Vz_F_pred	mL	11739.1412
Cl_F_pred	mL/min	43.7095
AUMClast	min*min*ug/mL	1800842.4000
AUMCINF_obs	min*min*ug/mL	2899563.3856
AUMC_%Extrap_obs	%	37.8926
AUMCINF_pred	min*min*ug/mL	3084999.8783
AUMC_%Extrap_pred	%	41.6259
MRTlast	min	334.4164
MRTINF_obs	min	436.0243
MRTINF_pred	min	449.4791
AUC0_30	min*ug/mL	0.0000
AUC0_40	min*ug/mL	0.0000
AUC0_60	min*ug/mL	0.0000

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=313-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 11

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	0.0000			0.0000	0.0000	
30.00	0.0000			0.0000	0.0000	
40.00	3.240			16.20	648.0	
60.00	5.990			108.5	5538.	
90.00	8.990			333.2	2.307e+004	
120.0	12.02			648.4	5.684e+004	
240.0	8.040			1852.	2.592e+005	
360.0 *	8.180	8.276	-0.09590	2825.	5.516e+005	1.000
480.0 *	6.290	6.145	0.1449	3693.	9.095e+005	1.000
600.0 *	4.510	4.563	-0.05287	4341.	1.253e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9954
Rsq_adjusted		0.9908
Corr_XY		-0.9977
No_points_lambda_z		3
Lambda_z	1/min	0.0025
Lambda_z_lower	min	360.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	279.4033
Tlag	min	30.0000
Tmax	min	120.0000
Cmax	ug/mL	12.0200
Cmax_D	ug/mL/mg	0.0401
Tlast	min	600.0000
Clast	ug/mL	4.5100
AUClast	min*ug/mL	4341.3500
AUCall	min*ug/mL	4341.3500
AUCINF_obs	min*ug/mL	6159.3028
AUCINF_D_obs	min*ug/mL/mg	20.5310
AUC_%Extrap_obs	%	29.5156
Vz_F_obs	mL	19633.4111
Cl_F_obs	mL/min	48.7068
AUCINF_pred	min*ug/mL	6180.6159
AUCINF_D_pred	min*ug/mL/mg	20.6021
AUC_%Extrap_pred	%	29.7586
Vz_F_pred	mL	19565.7078
Cl_F_pred	mL/min	48.5389
AUMClast	min*min*ug/mL	1252974.0000
AUMCINF_obs	min*min*ug/mL	3076551.0815
AUMC_%Extrap_obs	%	59.2734

AUMCINF_pred	min*min*ug/mL	3097930.0839
AUMC_%Extrap_pred	%	59.5545
MRTlast	min	288.6139
MRTINF_obs	min	499.4966
MRTINF_pred	min	501.2332
AUC0_30	min*ug/mL	0.0000
AUC0_40	min*ug/mL	16.2000
AUC0_60	min*ug/mL	108.5000

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=314-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 11

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		3.750			28.13	421.9	
30.00		7.880			115.4	2617.	
40.00		8.160			195.6	5431.	
60.00		9.860			375.8	1.461e+004	
90.00		13.06			719.6	4.112e+004	
120.0		16.04			1156.	8.762e+004	
240.0	*	12.78	12.45	0.3291	2885.	3.871e+005	1.000
360.0	*	7.380	7.472	-0.09176	4095.	7.306e+005	1.000
480.0	*	4.250	4.484	-0.2338	4793.	1.012e+006	1.000
600.0	*	2.800	2.691	0.1093	5216.	1.236e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9960
Rsq_adjusted		0.9939
Corr_XY		-0.9980
No_points_lambda_z		4
Lambda_z	1/min	0.0043
Lambda_z_lower	min	240.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	162.8814
Tlag	min	0.0000

Tmax	min	120.0000
Cmax	ug/mL	16.0400
Cmax_D	ug/mL/mg	0.0535
Tlast	min	600.0000
Clast	ug/mL	2.8000
AUClast	min*ug/mL	5215.6500
AUCall	min*ug/mL	5215.6500
AUCINF_obs	min*ug/mL	5873.6168
AUCINF_D_obs	min*ug/mL/mg	19.5787
AUC_%Extrap_obs	%	11.2021
Vz_F_obs	mL	12002.2201
Cl_F_obs	mL/min	51.0759
AUCINF_pred	min*ug/mL	5847.9331
AUCINF_D_pred	min*ug/mL/mg	19.4931
AUC_%Extrap_pred	%	10.8121
Vz_F_pred	mL	12054.9329
Cl_F_pred	mL/min	51.3002
AUMClast	min*min*ug/mL	1235586.7500
AUMCINF_obs	min*min*ug/mL	1784981.2110
AUMC_%Extrap_obs	%	30.7787
AUMCINF_pred	min*min*ug/mL	1763535.6685
AUMC_%Extrap_pred	%	29.9370
MRTlast	min	236.8999
MRTINF_obs	min	303.8981
MRTINF_pred	min	301.5656
AUC0_30	min*ug/mL	115.3500
AUC0_40	min*ug/mL	195.5500
AUC0_60	min*ug/mL	375.7500

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=315-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 10

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: User-specified lambda_z range, Log regression

User's lambda_z bounds: 240.00, 480.00

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	2.721			20.41	306.1	
30.00	8.883			107.4	2611.	
40.00	11.32			208.4	6207.	

60.00	17.85			500.1	2.144e+004	
90.00	23.38			1119.	6.907e+004	
120.0	26.37			1865.	1.481e+005	
240.0 *	21.03	20.83	0.1977	4708.	6.408e+005	1.000
360.0 *	8.343	8.502	-0.1591	6471.	1.124e+006	1.000
480.0 *	3.503	3.470	0.03293	7182.	1.405e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9997
Rsq_adjusted		0.9993
Corr_XY		-0.9998
No_points_lambda_z		3
Lambda_z	1/min	0.0075
Lambda_z_lower	min	240.0000
Lambda_z_upper	min	480.0000
HL_Lambda_z	min	92.8176
Tlag	min	0.0000
Tmax	min	120.0000
Cmax	ug/mL	26.3680
Cmax_D	ug/mL/mg	0.0879
Tlast	min	480.0000
Clast	ug/mL	3.5030
AUClast	min*ug/mL	7181.5775
AUCall	min*ug/mL	7181.5775
AUCINF_obs	min*ug/mL	7650.6553
AUCINF_D_obs	min*ug/mL/mg	25.5022
AUC_%Extrap_obs	%	6.1312

Vz_F_obs	mL	5250.8227
Cl_F_obs	mL/min	39.2123
AUCINF_pred	min*ug/mL	7646.2452
AUCINF_D_pred	min*ug/mL/mg	25.4875
AUC_%Extrap_pred	%	6.0771
Vz_F_pred	mL	5253.8511
Cl_F_pred	mL/min	39.2349
AUMClast	min*min*ug/mL	1404876.7500
AUMCINF_obs	min*min*ug/mL	1692847.0969
AUMC_%Extrap_obs	%	17.0110
AUMCINF_pred	min*min*ug/mL	1690139.7144
AUMC_%Extrap_pred	%	16.8781
MRTlast	min	195.6223
MRTINF_obs	min	221.2682
MRTINF_pred	min	221.0418
AUC0_30	min*ug/mL	107.4375
AUC0_40	min*ug/mL	208.4425
AUC0_60	min*ug/mL	500.1125

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=317-pfs

Date: 10/07/2011

Time: 09:48:13

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 9

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight
min	ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000	0.0000			0.0000	0.0000	
15.00	0.0000			0.0000	0.0000	
30.00	6.355			47.66	1430.	
40.00	9.037			124.6	4191.	
60.00	12.21			337.1	1.513e+004	
90.00	21.18			837.9	5.471e+004	
120.0 *	12.07	12.04	0.02577	1337.	1.050e+005	1.000
240.0 *	5.983	6.009	-0.02563	2420.	2.781e+005	1.000
360.0 *	3.004	2.998	0.006413	2959.	4.291e+005	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq	1.0000
Rsq_adjusted	0.9999

Corr_XY		-1.0000
No_points_lambda_z		3
Lambda_z	1/min	0.0058
Lambda_z_lower	min	120.0000
Lambda_z_upper	min	360.0000
HL_Lambda_z	min	119.6131
Tlag	min	15.0000
Tmax	min	90.0000
Cmax	ug/mL	21.1770
Cmax_D	ug/mL/mg	0.0706
Tlast	min	360.0000
Clast	ug/mL	3.0040
AUClast	min*ug/mL	2959.0025
AUCall	min*ug/mL	2959.0025
AUCINF_obs	min*ug/mL	3477.3885
AUCINF_D_obs	min*ug/mL/mg	11.5913
AUC_%Extrap_obs	%	14.9073
Vz_F_obs	mL	14887.4864
Cl_F_obs	mL/min	86.2716
AUCINF_pred	min*ug/mL	3476.2818
AUCINF_D_pred	min*ug/mL/mg	11.5876
AUC_%Extrap_pred	%	14.8802
Vz_F_pred	mL	14892.2261
Cl_F_pred	mL/min	86.2991
AUMClast	min*min*ug/mL	429125.0250
AUMCINF_obs	min*min*ug/mL	705199.3926
AUMC_%Extrap_obs	%	39.1484
AUMCINF_pred	min*min*ug/mL	704609.9809
AUMC_%Extrap_pred	%	39.0975

MRTlast	min	145.0235
MRTINF_obs	min	202.7957
MRTINF_pred	min	202.6907
AUC0_30	min*ug/mL	47.6625
AUC0_40	min*ug/mL	124.6225
AUC0_60	min*ug/mL	337.0925

Input File: Workbook - [C:\Er...\Data HM13424.xls]

Subject_ID=318-pfs

Date: 10/07/2011

Time: 09:48:14

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Version 5.1 Build 200607251915

Core Version 18Apr2006

Settings

Model: Plasma Data, Extravascular Administration

Number of nonmissing observations: 8

Dose time: 0.00

Dose amount: 300.00

Calculation method: Linear Trapezoidal with Linear Interpolation

Weighting for lambda_z calculations: Uniform weighting

Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time	Conc.	Pred.	Residual	AUC	AUMC	Weight

min		ug/mL	ug/mL	ug/mL	min*ug/mL	min*min*ug/mL	
0.0000		0.0000			0.0000	0.0000	
15.00		0.0000			0.0000	0.0000	
30.00		0.0000			0.0000	0.0000	
40.00		3.590			17.95	718.0	
90.00		11.09			385.0	2.926e+004	
240.0	*	16.79	17.25	-0.4550	2476.	4.063e+005	1.000
360.0	*	11.21	10.77	0.4408	4156.	8.903e+005	1.000
600.0	*	4.144	4.200	-0.05578	5998.	1.673e+006	1.000

*) Starred values were included in the estimation of Lambda_z.

Final Parameters

Rsq		0.9976
Rsq_adjusted		0.9952
Corr_XY		-0.9988
No_points_lambda_z		3
Lambda_z	1/min	0.0039
Lambda_z_lower	min	240.0000
Lambda_z_upper	min	600.0000
HL_Lambda_z	min	176.6614
Tlag	min	30.0000
Tmax	min	240.0000
Cmax	ug/mL	16.7900
Cmax_D	ug/mL/mg	0.0560
Tlast	min	600.0000
Clast	ug/mL	4.1440
AUClast	min*ug/mL	5998.4300

AUCall	min*ug/mL	5998.4300
AUCINF_obs	min*ug/mL	7054.6054
AUCINF_D_obs	min*ug/mL/mg	23.5154
AUC_%Extrap_obs	%	14.9714
Vz_F_obs	mL	10838.3911
Cl_F_obs	mL/min	42.5254
AUCINF_pred	min*ug/mL	7068.8220
AUCINF_D_pred	min*ug/mL/mg	23.5627
AUC_%Extrap_pred	%	15.1424
Vz_F_pred	mL	10816.5932
Cl_F_pred	mL/min	42.4399
AUMClast	min*min*ug/mL	1672890.0000
AUMCINF_obs	min*min*ug/mL	2575781.1328
AUMC_%Extrap_obs	%	35.0531
AUMCINF_pred	min*min*ug/mL	2587934.5180
AUMC_%Extrap_pred	%	35.3581
MRTlast	min	278.8880
MRTINF_obs	min	365.1205
MRTINF_pred	min	366.1055
AUC0_30	min*ug/mL	0.0000
AUC0_40	min*ug/mL	17.9500
AUC0_60	min*ug/mL	119.7500

VITA

Eric S. Edwards

Eric S. Edwards is the Co-founder and Chief Science Officer at Intellijject, Inc., a pharmaceutical company in Richmond, VA developing novel medicines for the treatment of a variety or chronic and acute-care diseases. In 2011, the Company announced tentative Food and Drug Administration approval of their first product, e-cue™, an epinephrine auto-injector for life-threatening allergic emergencies (anaphylaxis). In November 2009, Intellijject signed a commercial licensing agreement with Sanofi for commercialization of e-cue™ in the United States and Canada. The novel drug/device combination platforms that Mr. Edwards and his identical twin brother engineer, Evan, invented are being incorporated into the development of several life-saving or life-enhancing medicines at Intellijject.

Mr. Edwards is named on over 25 issued and 75 pending patent applications domestically and abroad and is a published author on more than half a dozen scientific publications. At Intellijject, he is responsible for overseeing the Company's innovative pharmaceutical research and development pipeline, managing the company's clinical program strategy, assisting with pharmaceutical development and regulatory affairs efforts, and leading all aspects of medical affairs. Prior to joining Intellijject, Mr. Edwards completed two years of formal medical school education at Virginia Commonwealth University and Step I of the United States Medical

Licensing Examination. Prior to this, he obtained a B.S. in Biology, Magna Cum Laude, with honors from VCU.

Mr. Edwards has won numerous awards recognizing his entrepreneurial spirit, innovative approach to pharmaceutical product development and dedication to research and community service, including being recognized as one of the top collegiate inventors by the National Collegiate Inventors and Innovators Alliance, being named one of the Top 25 Entrepreneurs of the Past 25 Years in Richmond, VA and receiving the Charles T. Rector and Thomas W. Rorrer, Jr. Dean's Award for Excellence in Graduate Pharmaceutical Science Research at VCU. He currently lives in Richmond, Virginia, U.S.A. with his wife, Autum, and three children.