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Therapeutic Drug Monitoring of Factor VIII Prophylaxis Using Its Plasma Coagulant Activity and Global Hemostasis Biomarkers: A Pharmacokinetic/Pharmacodynamic Pilot Study

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Therapeutic Drug Monitoring of Factor VIII Prophylaxis Using Its

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A Pharmacokinetic/Pharmacodynamic Pilot Study

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

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Dedication

This dissertation is dedicated to my beloved parents, Abdullah and Dakila, and my treasured siblings, Firyal, Mohammad, Hussain, Ali and Sarah. Their boundless love, faith and support paved the way to achieve my dreams. For their sincere prayers, I owe all the blessings that I have in my life.

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List of Abbreviations

Abbreviation	Expansion
Al(OH)3	Aluminum hydroxide
ANOVA	Analysis of variance
aPCC	Activated prothrombin complex concentrate
aPTT	Activated partial thromboplastin time
ASGPR	Asialoglycoprotein receptor
AUC_{∞}	Total area under the curve
AUC_{extra}	Extrapolated area under the curve from last point to infinity
CA	Compartmental analysis
Ca^{+2}	Calcium ions
$CaCl_2$	Calcium chloride
CAD	Coronary artery disease
CEM	Clot elastic modulus
CL	Clearance
CL_{tot}	Total body clearance
C_{max}	Maximum concentration
CNS	Central nervous system
CT	Clotting time
CTF	Clot formation time

Abbreviation	Expansion
CVCCD	Central Virginia Center for Coagulation Disorders
DNA	Deoxyribonucleic acid
DVT	Deep vein thrombosis
E_0	Baseline effect
EACA	Epsilon-aminocaproic acid
EC_{50}	Half the maximum effective concentration
E_{max}	Maximum effect
FII	Factor II or prothrombin
FIIa	Activated factor II or thrombin
FV	Factor V
FVa	Activated factor V
FVII	Factor VII
FVIIa	Activated factor VII
FVIII	Factor VIII
FVIII:Ag	Factor VIII antigen
FVIII:C	Factor VIII coagulant activity
FVIIIa	Activated factor VIII
FIX	Factor IX
FIXa	Activated factor IX
FX	Factor X
FXa	Activated factor X
FXI	Factor XI

Abbreviation	Expansion
FXIa	Activated factor XI
FXII	Factor XII
FXIIa	Activated factor XII
FOT	Force onset time
f_u	Unbound fraction
GCRC	General Clinical Research Center
GPIIb/IIIa	Glycoprotein IIb/IIIa
h	Hour
HAV	Hepatitis A virus
HAS	Hemostasis Analysis System
HBV	Hepatitis B virus
HCV	Hepatitis C virus
Hemophilia	Hemophilia A
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HMWK	High molecular weight kininogen
HSPG	Heparan-sulfate proteoglycans
HTC	Hemophilia treatment center
ID	Identification
IRB	Institutional Review Board
IU	International unit
IU dL^{-1}	International unit per deciliter

Abbreviation	Expansion
IU kg^{-1}	International unit per kilogram
IU mg^{-1}	International unit per milligram
IU mL^{-1} min^{-1}	International unit per milliliter per minute
IV	Intravenous
K	Kinetics-time
kdynes	Kilodynes
kdynes cm^{-2}	Kilodynes per square centimeter
kdynes dL IU^{-1}	Kilodynes deciliter per international unit
kdynes dL IU^{-1} cm^{-2}	Kilodynes deciliter per international unit per square centimeter
kg	Kilogram
L^{-1}	Per Liter
LDLR	Low density lipoprotein receptor
LRP1	Low density lipoprotein receptor-related protein-1
M	Molar
MA	Maximum amplitude
MASAC	Medical and Scientific Advisory Council
MCF	Maximum clot firmness
mg dL^{-1}	Milligram per deciliter
min	Minute
mm	Millimeter
mg	Milligram
mL	Millimeter

Abbreviation	Expansion
mL h^{-1} kg^{-1}	Milliliter per hour per kilogram
mL kg^{-1}	Milliliter per kilogram
MOA	Mechanism of action
MRT	Mean residence time
MSC	Model selection criterion
n	Hill coefficient
NCA	Non-compartmental analysis
NSAID	Non-steroidal anti-inflammatory drug
PCF	Platelet contractile force
PD	Pharmacodynamic
pd FVIII	Plasma-derived factor VIII
PK	Pharmacokinetic
PT	Prothrombin
R	Reaction-time
r^2	Coefficient of determination
rFVIIa	Activated recombinant factor VII
rFVIII	Recombinant factor VIII
RBC	Red blood cell
ROTEM	Thromboelastometry
SD	Standard deviation
sec	Second
sec dL IU^{-1}	Second deciliter per international unit

Abbreviation	Expansion
$t_{1/2}$	Terminal half-life
TDM	Therapeutic drug monitoring
TEG	Thromboelastography
TF	Tissue factor
TGT	Thrombin generation time
t_{max}	Time to maximum concentration
VCAL	Virginia Commonwealth University Coagulation Laboratory
VCU	Virginia Commonwealth University
V_{ss}	Volume of distribution at steady-state
vWD	von Willebrand disease
vWF	von Willebrand factor
WFH	World Federation of Hemophilia
WHO	World Health Organization

Abstract

THERAPEUTIC DRUG MONITORING OF FACTOR VIII PROPHYLAXIS USING ITS PLASMA COAGULANT ACTIVITY AND GLOBAL HEMOSTASIS BIOMARKERS: A PHARMACOKINETIC/PHARMACODYNAMIC PILOT STUDY

By:

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I. Background:

The current clinical practice of factor VIII (FVIII) prophylaxis revolves around converting patients with severe hemophilia A, hereafter simply referred to as hemophilia, phenotype (defined as plasma factor VIII coagulant activity [FVIII:C] <1 IU dL^{-1}) to moderate

hemophilia phenotype (defined as plasma FVIII:C from 1–5 IU dL^{-1}). However, a wide inter-individual variation in bleeding tendency is observed despite changes in plasma FVIII:C (pharmacokinetic [PK] changes). Therefore, monitoring FVIII prophylaxis by global hemostasis biomarkers (pharmacodynamic [PD] response) can potentially be beneficial.

II. Objective:

To conduct appropriate PK/PD modeling using plasma FVIII:C and global hemostasis (platelet function and blood viscoelastic) biomarkers in severe hemophilia.

III. Methods:

Nine non-bleeding severe hemophiliacs (plasma FVIII:C <1 IU dL^{-1}) with variant bleeding tendency (5 frequent bleeders and 4 infrequent bleeders) were infused with a recombinant factor FVIII (rFVIII) prophylactic dose (mean = 32.1 international units per kilogram [IU kg^{-1}]). Blood was collected at baseline and 0.5-, 1-, 2-, 4-, 8-, 12-, 24- and 48-hours (h) post-dose for plasma FVIII:C, platelet function (platelet contractile force [PCF], clot elastic modulus [CEM] and force onset time [FOT]) and blood viscoelastic (reaction-time [R], kinetics-time [K] and maximum amplitude [MA]) biomarkers and activated partial thromboplastin time (aPTT). Non-compartmental analysis (NCA) was performed using standard methods. Compartmental analysis (CA) and PK/PD modeling were performed by non-linear regression. Correlation and analysis of variance (ANOVA) were used to explore the role of clinically relevant modifiers of bleeding tendency, as appropriate. ANOVA was used to assess inter-group differences in pertinent PK and PD parameters. *P* value <0.05 significance level was pre-specified for all statistical tests.

IV. Results:

Mean (±SD) volume of distribution at steady state (V_{ss}), total clearance (CL_{tot}) and terminal half-life ($t_{1/2}$) from NCA were 40.5 (±11.2) milliliter per kilogram (mL kg^{-1}), 2.9 (±1.2) milliliter per hour per kilogram (mL h^{-1} kg^{-1}) and 11.6 (±6.2) h, respectively. Mean (±SD) V_{ss} and CL_{tot} from the one-compartment body model (CA) were 39.6 (±8.9) mL kg^{-1} and 3.1 (±1.3) mL h^{-1} kg^{-1}, respectively. The mean (±SD) baseline effect (E_0) and slope from the PK/PD linear modeling were: for aPTT, 48.9 (±4.4) seconds (sec) and -0.025 (±0.009) second deciliter per international unit (sec dL IU^{-1}), respectively; for PCF, 0.3 (±0.3) kilodynes (kdynes) and 0.008 (±0.004) kilodynes deciliter per international unit (kdynes dL IU^{-1}), respectively; and for CEM, 0.0 (±0.0) kilodynes per square centimeter (kdynes cm^{-2}) and 0.032 (±0.016) kilodynes deciliter per international unit per square centimeter (kdynes dL IU^{-1} cm^{-2}), respectively. The mean (±SD) maximum effect (E_{max}) and half the maximum effective concentration (EC_{50}) from the PK/PD sigmoidal E_{max} model were: for FOT, 70.1 (±16.9) % reduction and 87.8 (±31.4) IU dL^{-1} for FOT, respectively; for R, 74.9 (±26.0) % reduction and 68.5 (±28.4) IU dL^{-1}, respectively; and for K, 73.2 (±36.4) % reduction and 67.2 (±29.0) IU dL^{-1}, respectively. MA was not PK/PD modeled due to its low sensitivity.

V. Conclusions:

Plasma FVIII:C remained ≥1 IU dL^{-1} over the prophylactic interval. FOT and R were the most sensitive biomarkers at lower plasma FVIII:C. PCF and CEM were more sensitive than K and aPTT at lower plasma FVIII:C. MA was the least sensitive biomarker. Correlation and inter-group differences did not reach statistical significance (small sample size). These results may be used to assess risk of bleeding and dose-optimize FVIII prophylaxis in severe hemophilia.

CHAPTER 1. BACKGROUND

1.1. HEMOPHILIA A:

1.1.1. Definition and Epidemiology of Hemophilia A:

Hemophilia A or classic hemophilia, **hereafter simply referred to as hemophilia**, is a coagulation disorder that is caused by a recessive X-linked deficiency of FVIII.[1–3] The mutant X-chromosome is expressed in males and carried in females, making hemophilia a disease of males with carrier females.[1–3] In 2009, the prevalence of hemophilia was 115,204 males in 105 countries.[4] The mean global per capita usage of FVIII, the blood coagulation factor replacement therapy used in hemophilia, was 2.02 international units (IU) in 2009.[4] The total annual global FVIII consumption was 7,333,984,513 IU for the same year.[4] Hemophilia is found in all ethnic groups worldwide.[1]

1.1.2. Pathophysiology of Hemophilia A:

Males with hemophilia have the defective allele on the X-chromosome without a matching allele on the Y-chromosome while carrier females have only one defective allele.[1] Sons of a father with hemophilia would be normal if the mother is not a carrier; however, daughters would be mandatory carriers (Figure 1.1).[1] If the father does not have hemophilia, sons and daughters of a carrier mother would have 50% chance of being hemophiliacs and carriers, respectively (Figure 1.1).[1] Therefore, there is a skipped generation inheritance pattern with hemophilia in which carrier females do not exhibit the disease but can transmit the defective

X-chromosome to the next male generation, who exhibit the disease.[5] Sons of a father with hemophilia and a carrier mother have 50% chance of being hemophiliac whereas daughters would be either hemophiliacs (very rare) or carriers (Figure 1.1).[1,6]

The pathophysiology of hemophilia on the molecular level includes mutation in the FVIII gene that encodes FVIII.[2] Several mutations in the FVIII gene have been documented, including point mutations, deletions and insertions.[2] The most common mutation in the FVIII gene is intron 22 inversion.[2] The intron 22 inversion accounts for about 50% of severe hemophilia abnormalities.[2] Another common mutation in the FVIII gene is intron 1 inversion.[2] The intron 1 inversion accounts for about 5% of severe hemophilia abnormalities.[2]

Figure 1.1. Inheritance of Hemophilia

Case 1

		Hemophiliac Male X’	Hemophiliac Male Y
Normal Female	X	Carrier Female X’X	Normal Male XY
Normal Female	X	Carrier Female X’X	Normal Male XY

Case 2

		Normal Male X	Normal Male Y
Carrier Female	X’	Carrier Female X’X	Hemophiliac Male X’Y
Carrier Female	X	Normal Female XX	Normal Male XY

Case 3

		Hemophiliac Male X’	Hemophiliac Male Y
Carrier Female	X’	Hemophiliac Female X’X’	Hemophiliac Male X’Y
Carrier Female	X	Carrier Female X’X	Normal Male XY

X’ is the defective chromosome.

1.1.3. Diagnosis of Hemophilia A:

Hemophilia is suspected due to a recognized family history of coagulation disorders or after the occurrence of unusual bleeding.[1,2] The clinical manifestations of hemophilia (Table 1.1) are typically first detected upon an excessive unusual bleeding.[7–9] Upon an initial diagnosis of hemophilia, the diagnosis is confirmed in clinical settings by appropriate laboratory tests (Table 1.2).[1,2,9] In hemophilia, laboratory tests indicate a low FVIII plasma level, measured as FVIII coagulant activity (FVIII:C) in plasma, and a prolonged activated partial thromboplastin time (aPTT); however, prothrombin time (PT), von Willebrand factor (vWF) plasma level and platelet count are normal.[1,2,9] Genetic testing for hemophilia is recommended for a more confirmatory diagnosis, especially for carrier females.[1,2] Upon successful diagnosis of hemophilia with unknown family history, other family members should be screened.[1]

Table 1.1. Clinical Manifestations of Hemophilia[7–9]

Signs and Symptoms
• Ecchymosis, erythema and swelling
• Hemarthrosis, arthropathy and joints deformity
• Musculoskeletal hemorrhage and impairment
• Genitourinary hemorrhage and hematuria
• Gastrointestinal hemorrhage and melena
• Intracranial hemorrhage
• Bleeding after surgeries and dental procedures
• Limited mobility, nerve palsy and pain

Table 1.2. Laboratory Testing of Hemophilia[1,2,9]

Test	**Value**
Plasma FVIII:C	Low
aPTT	Prolonged
PT	Normal
vWF plasma level	Normal
Platelet count	Normal

1.1.4. Classification of Hemophilia A:

Hemophilia is classified into three phenotypes: severe, moderate and mild (Table 1.3).[1,3,10] This classification is based on plasma FVIII:C.[1,3,10] Severe hemophilia phenotype is defined as plasma FVIII:C <1 international unit per deciliter (IU dL^{-1}).[1,3,10] Moderate hemophilia phenotype is defined as plasma FVIII:C from 1–5 IU dL^{-1}.[1,3,10] Mild hemophilia phenotype is defined as plasma FVIII:C >5–40 IU dL^{-1}.[1,3,10] The clinical presentations of hemophilia phenotypes are assumed to match the measured plasma FVIII:C.[1,10] In case of severe FVIII deficiency (plasma FVIII:C <1 IU dL^{-1}), severe clinical presentations that include spontaneous bleeding in joints and muscles, and bleeding due to injuries, accidents, surgeries and dental procedures are observed.[1,10] In case of moderate FVIII deficiency (plasma FVIII:C from 1–5 IU dL^{-1}), moderate clinical presentations that include bleeding in joints and muscles due to insignificant injuries, and bleeding due to accidents, surgeries and dental procedures are observed.[1,10] In case of mild FVIII deficiency (plasma FVIII:C >5–40 IU dL^{-1}), mild clinical presentations that include absence of spontaneous bleeding in joints and muscles, and possible bleeding due to significant injuries, accidents, surgeries and dental procedures are observed.[3,10]

Table 1.3. Classification of Hemophilia[1,3,10]

Phenotype	**Plasma FVIII:C** (IU dL^{-1})	**Clinical Presentations**
Severe	<1	• Spontaneous bleeding in joints and muscles; bleeding due to injuries, accidents, surgeries and dental procedures
Moderate	1–5	• Bleeding in joints and muscles due to insignificant injuries; bleeding due to accidents, surgeries and dental procedures
Mild	>5–40	• No spontaneous bleeding in joints and muscles; possible bleeding due to significant injuries, accidents, surgeries and dental procedures

1.1.5. Management of Hemophilia A:

Hemophilia management necessitates a comprehensive multidisciplinary healthcare.[11] Hemophilia patients are best treated in a specialized center, usually referred to as hemophilia treatment center (HTC), with trained healthcare professionals and appropriate services.[11] Healthcare professionals involved in an HTC include hematologists, nurses, orthopedic surgeons, physical therapists, pharmacists, dentists, psychologists, social workers and genetic counselors.[11] Healthcare services required in an HTC includes clinical laboratory, radiology and pharmacy.[11]

The primary goal in hemophilia is to prevent or treat bleeding episodes in order to prevent or reduce related complications, e.g., joint damage (arthropathy).[12] The main pharmacotherapy for bleeding episodes in hemophilia is FVIII replacement therapy.[12] FVIII is a parenteral product, and therefore it requires extensive training to learn how to administer FVIII at home.[13] Family members may learn how to infuse FVIII for younger children with hemophilia.[13] Older children and adults with hemophilia may also learn how to self-infuse FVIII.[13] In case of difficult venous access, home nursing support is available.[13] Due to risk of transmitting viral infection through FVIII products, routine immunizations, including hepatitis A virus (HAV) and hepatitis B virus (HBV), should be provided to hemophiliacs.[14]

An extended use of FVIII therapy is associated with development of FVIII inhibitory antibodies.[12] FVIII inhibitory antibodies neutralize the infused FVIII therapy, and therefore FVIII therapy becomes ineffective.[12] In 2009, the prevalence of clinically identified FVIII inhibitory antibodies in hemophilia was 5,013 (out of 115,204) males.[4] Recombinant FVIII (rFVIII) products have a higher risk of developing FVIII inhibitory antibodies compared to

plasma-derived FVIII (pdFVIII) products.[12] The pharmacotherapy for bleeding episodes in the presence of FVIII inhibitory antibodies requires administration of a hemostatic bypassing agent that is able to initiate blood clotting without the presence of FVIII.[12] Current commonly used hemostatic bypassing agents include activated prothrombin complex concentrate (aPCC) and activated recombinant factor VII (rFVIIa).[12]

The pharmacotherapy for bleeding episodes in hemophilia also includes 1-desamino-8-D-arginine vasopressin (desmopressin), antifibrinolytics and fibrin sealants.[12] Desmopressin is a synthetic analog of vasopressin, the antidiuretic hormone.[12] Desmopressin increases plasma FVIII:C as well as plasma level of vWF (FVIII carrier and protector against early degradation by plasma proteases).[12] Therefore, desmopressin can be used in mild and moderate bleeding episodes of hemophilia.[12] Desmopressin can actually cause two-fold to six-fold elevation in plasma FVIII:C.[12] Desmopressin mechanism of action (MOA) is poorly understood.[12] However, desmopressin is hypothesized to release FVIII and vWF from their endogenous storage sites.[12] Desmopressin is administered intravenously.[12]

Antifibrinolytics, e.g., tranexamic acid and epsilon-aminocaproic acid (EACA), inhibit clot lysis. [12] Therefore, antifibrinolytics are used as an adjunctive therapy in hemophilia bleeding episodes.[12] Antifibrinolytics are particularly useful in mucosal surface bleeding and oral surgery.[12] Antifibrinolytics are administered topically, orally or intravenously.[12]

Fibrin sealants are a mixture of thrombin and fibrinogen.[12] Thrombin in fibrin sealants converts fibrinogen to an insoluble fibrin clot.[12] Therefore, fibrin sealants stop hemophilia bleeding episodes in oral and maxillofacial surgery as well as in orthopedic surgery.[12] Fibrin sealants are applied topically.[12]

Pain, acute and chronic, is common in hemophilia.[12] Acute pain is caused by joint bleeding (hemarthrosis).[12] Repeated hemarthrosis may progress to chronic arthropathy that causes chronic pain.[12] Therefore, treatment of bleeding is essential for pain management. There are no specific guidelines for pain management in hemophilia.[12] Nevertheless, aspirin, a non-steroidal anti-inflammatory drug (NSAID), should be avoided due to its antiplatelet effect.[12] Other NSAIDs also have antiplatelet effect.[12] However, NSAIDs are not clinically proven to increase the bleeding tendency in hemophilia.[12] Acetaminophen is considered safe but not specifically effective.[12] Narcotics can be used in severe pain.[12] Surgical intervention and physical therapy may help to control pain in hemophilia.[15]

1.2. FACTOR VIII:

1.2.1. Factor VIII Physiology:

FVIII is a critical blood coagulation factor. It is a complex glycoprotein consisting of 2,351 amino acids.[1] FVIII is considered one of the largest blood coagulation factors.[1] FVIII is produced mainly by hepatocytes; however, it is produced in small amounts by sinusoidal endothelial cells, glomerular cells, tubular endothelial cells and lymphatic tissues.[1,16] FVIII is the least stable blood coagulation factor.[1] It is highly susceptible to plasma proteolytic degradation facilitated through phospholipid-binding proteases, e.g., activated protein C and activated factor X (FXa).[1,17] FVIII is also susceptible to cellular uptake into hepatocytes and macrophage-like Kupffer cells in the liver.[17] FVIII cellular uptake is facilitated by a number of receptors and other cell membrane components expressed mainly on hepatocytes and to some extent on Kupffer cells, including low density lipoprotein receptor (LDLR), LDLR-related protein-1 (LRP1), heparan-sulfate proteoglycans (HSPG), megalin, asialoglycoprotein receptor

(ASGPR) and unidentified carbohydrate receptors.[17] In plasma, FVIII circulates with a tight, non-covalent bond to vWF, which acts as its carrier.[1,17] The biological functions of vWF as FVIII carrier include FVIII heterodimeric structure stabilization, FVIII plasma proteolytic degradation prevention and FVIII hepatic cellular uptake inhibition.[17]

1.2.2. Factor VIII Replacement Therapy:

FVIII replacement therapy in hemophilia revolves around three clinical strategies: primary prophylaxis, secondary prophylaxis and on-demand treatment (Table 1.4).[18] Primary FVIII prophylaxis is a long-term therapy on a continuous basis initiated either 'after the first episode of joint bleeding and before 2 years of age' or 'before 2 years of age without any joint bleeding episode.'[18] Secondary FVIII prophylaxis may consist of either long-term or short-term prophylaxis.[18] Long-term secondary FVIII prophylaxis is a continuous therapy initiated either 'after two episodes of joint bleeding' or 'at >2 years of age.'[18] Short-term FVIII prophylaxis is an intermittent therapy initiated after frequent bleeding episodes.[18] On-demand FVIII treatment is a therapy initiated only upon the occurrence of bleeding and until it stops.[18]

Table 1.4. Clinical Strategies for FVIII Replacement Therapy[18]

Strategy	FVIII Replacement Therapy
Primary prophylaxis	
Long-term (continuous)	
By bleeding	• After the first joint bleeding and before the 2 years of age
By age	• Before the 2 years of age without any joint bleeding episode
Secondary prophylaxis	
Long-term (continuous)	
By bleeding	• After two episodes of joint bleeding
By age	• At >2 years of age
Short-term (intermittent)	• After frequent bleeding episodes
On-demand treatment	• Upon the occurrence of bleeding

1.2.3. Factor VIII Products:

FVIII products are either plasma-derived or recombinant proteins. There are various pdFVIII products licensed in the United States (Table 1.5).[19–24] The first pdFVIII product was introduced in the 1960s.[25] Isolated from thousands of blood donors, pdFVIII products carry a potential risk of transmitting infection.[3,12,25] Several reports of viral transmission, especially human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in the 1980s, were documented.[12,25,26] However, current technologies ensure the safety of pdFVIII products through vigorous purification and viral inactivation procedures.[12,25,26]

Table 1.5. pdFVIII Products Licensed in the United States

Brand Name	Manufacturer or Distributer	Site
Alphanate[19]	Grifols Biologicals Inc	Los Angeles, CA
Hemofil M[20]	Baxter Healthcare Corp	Westlake Village, CA
Humate-P[21]	CSL Behring LLC	Kankakee, IL
Koāte-DVI[22]	Talecris Biotherapeutics Inc	Research Triangle Park, NC
Monarc-M[23]	Baxter Healthcare Corp	Westlake Village, CA
Monoclate-P[24]	CSL Behring LLC	Kankakee, IL

According to FVIII:C content per total protein milligram (mg), pdFVIII products are classified into four classes: low-, intermediate-, high- and ultrahigh-purity (Table 1.6).[25] Single-donor-cryoprecipitate is the only existing low-purity pdFVIII product.[25] It contains FVIII:C <5 international units per milligram (IU mg^{-1}) of protein.[25] Single-donor-cryoprecipitate is no longer used whenever other FVIII products are available due to absence of viral inactivation and purification procedures throughout its production.[25] Intermediate-purity pdFVIII products contain FVIII:C between 1–10 IU mg^{-1} of protein.[25] High-purity pdFVIII products contain FVIII:C between 50–1,000 IU mg^{-1} of protein.[25] Ultrahigh-purity pdFVIII products contain FVIII:C of 3,000 IU mg^{-1} of protein.[25]

Table 1.6. pdFVIII Products Classification[25]	
Purity	**FVIII:C Content** (IU mg^{-1} of Protein)
Low	<5
Intermediate	1–10
High	50–1,000
Ultrahigh	3,000

Several rFVIII products are licensed in the United States (Table 1.7).[27–32] The first rFVIII product was produced in 1984.[12,33] Utilizing mammalian cells transfected with human FVIII gene, rFVIII products are produced from advanced deoxyribonucleic acid (DNA) technologies.[12,33] Since rFVIII products are not derived from blood donors, they carry a minimal risk of infection transmission.[12,33] Therefore, rFVIII products are generally preferred over pdFVIII products whenever they are available. There is still a low risk of viral infection, theoretically, with some rFVIII products that contains human albumin or utilize it throughout their production.[25,33] Therefore, rFVIII products undergo viral inactivation and purification procedures although HIV and HCV transmission have never been documented with the use of rFVIII products.[12,25,33]

Table 1.7. rFVIII Products Licensed in the United States		
Brand Name	**Manufacturer or Distributer**	**Site**
Advate[27]	Baxter Healthcare Corp	Westlake Village, CA
Helixate FS[28]	CSL Behring LLC	Kankakee, IL
Kogenate FS[29]	Bayer HealthCare LLC	Tarrytown, NY
Recombinate[30]	Baxter Healthcare Corp	Westlake Village, CA
ReFacto[31]	Wyeth Pharmaceuticals Inc	Philadelphia, PA
Xyntha[32]	Wyeth Pharmaceuticals Inc	Philadelphia, PA

Based on utilization of human albumin in the cell culture and type of the stabilizer used, rFVIII products are classified into three classes: first-, second- and third-generation.[12,26,33] First-

generation FVIII products utilize human albumin in their cell culture and use it as a stabilizer.[12,33] Second-generation FVIII products are stabilized using sucrose.[12,33] However, they utilize human albumin in their cell culture.[12,33] Third-generation FVIII products are stabilized using sucrose or trehalose.[33] They do not utilize human albumin.[12,33]

Table 1.8. rFVIII Products Licensed in the United States[12,33]

Generation	Human Albumin in the Cell Culture	Stabilizer
First	Yes	Human albumin
Second	Yes	Sucrose
Third	No	Sucrose or trehalose

1.2.4. Factor VIII Dosing:

Current FVIII prophylactic dosing revolves around achieving a trough plasma FVIII:C ≥1 IU dL^{-1}.[3] For this purpose, the recommended FVIII routine prophylactic dose ranges from 25–40 international units per kilogram (IU kg^{-1}) three times per week.[3] The FVIII routine prophylactic dose may be modified empirically. For treatment of acute bleeding episodes, a FVIII loading dose, expressed in IU kg^{-1}, is calculated as 50% of the required elevation in plasma FVIII:C, expressed in IU dL^{-1} (Equation 1.1).[3,34]

$$\text{FVIII Loading Dose} = \frac{(\text{Desired FVIII:C} - \text{Baseline Plasma FVIII:C})}{2} \quad \text{...(Equation 1.1)}^{[3,34]}$$

Equation 1.1 assumes that each 1 IU kg^{-1} of the FVIII loading dose will elevate plasma FVIII:C by 2 IU dL^{-1}.[34] Table 1.9 shows the recommended guidelines for the desired plasma FVIII:C and the duration of FVIII therapy in different acute bleeding types.[25,34] Since the baseline plasma FVIII:C is usually very low, it may be excluded from Equation 1.1.[34] A FVIII maintenance dose is calculated as 50% of the FVIII loading dose and given every 12-hours (h) to

maintain the desired plasma FVIII:C over the recommended duration of FVIII therapy.[34] The recommended FVIII routine prophylactic dose (25–40 IU dL^{-1} three times per week) should be resumed after the treatment of acute episodes.

Table 1.9. Recommended Guidelines for FVIII Therapy in Acute Bleeding[25,34]

Bleeding Site or Type	**Plasma FVIII:C** (IU dL^{-1})	**Duration of FVIII Therapy** (Day)
Mucosa	30	1–2
Joint	50	Up to 7
Muscle	50	Up to 7
Gastrointestinal	50–100	10–14
Genitourinary	50–100	10–14
Central nervous system (CNS)	50–100	10–14
Trauma	50–100	Up to 14
Surgery	50–100	Up to 14

1.2.5. Factor VIII Pharmacokinetic Characteristics:

FVIII follows linear pharmacokinetic (PK) characteristics.[3] After a single short infusion of FVIII, the decline in the terminal β phase (disposition part) of the plasma FVIII:C-time profile exhibits a monoexponetial and a linear pattern on the linear and the logarithmic scale, respectively, thereby indicating a first-order kinetics and a linear PK.[3] A continuous infusion of FVIII produces a sustained plasma FVIII:C over the infusion duration that is proportional to FVIII infusion rate, thus demonstrating a linear PK.[3] Standard FVIII PK parameters, including volume of distribution at steady-state (V_{ss}), total clearance (CL_{tot}), terminal half-life ($t_{1/2}$), and mean residence time (MRT) are constant among different doses of FVIII, therefore representing dose-independent properties and a linear PK.[3] FVIII PK linearity was established for a dose range from 25–100 IU kg^{-1}.[3]

Standard FVIII PK parameters from representative single short infusion PK studies of FVIII in hemophilia are shown in Table 1.10.[3,35–40] FVIII:C V_{ss} ranges from 43–57 milliliters per kilogram (mL kg^{-1}).[3,35–40] Therefore, FVIII remains in the vascular system with only a small fraction distributed extravascularly.[3] FVIII:C CL_{tot} ranges from 2.4–3.4 milliliters per hour per kilogram (mL h^{-1} kg^{-1}).[3,35–40] FVIII clearance (CL) pathways have not been extensively investigated. FVIII is cleared by plasma phospholipid-binding proteases.[17] FVIII is also cleared by cellular uptake into hepatocytes and macrophage-like Kupffer cells.[17] FVIII is a large glycoprotein; therefore, it does not undergo renal clearance.[41] FVIII:C $t_{1/2}$ ranges from 11–16 h.[3,35–40] FVIII:C MRT ranges from 14–21 h.[3,35–40] FVIII unbound fraction (f_u) in plasma has not been comprehensively studied.[42] The plasma ratio of FVIII to vWF is 1:50.[42] Since FVIII circulates in plasma non-covalently bound to vWF, vWF may theoretically bind all FVIII molecules in plasma.[42] Consequently, a negligible FVIII f_u may remain in plasma that is rapidly cleared by plasma proteolytic degradation or by hepatic cellular uptake.

Table 1.10. Previously Reported Standard FVIII PK Parameters[3]

Study	V_{ss} (mL kg^{-1})	CL_{tot} (mL h^{-1} kg^{-1})	$t_{1/2}$ (h)	MRT (h)
pdFVIII				
Smith et al.[35]	44	3.4	14	14
Schwartz et al.[36]	57	3.2	14	18
Kasper et al.[37]	49	2.5	15	21
Björkman et al.[38]	54	2.9	14	19
Knevelman et al.[39]	43	2.4	14	20
Fijnvandraat et al.[40]	43	2.9	11	16
rFVIII				
Schwartz et al.[36]	51	2.5	16	21
Fijnvandraat et al.[40]	45	3.2	11	16

Variability in FVIII PK characteristics has been identified.[3,43–47] Critical factors that contribute to this variability include vWF, blood group, age, bodyweight, medical states, FVIII

inhibitory antibodies, FVIII assays and FVIII products (pdFVIII versus rFVIII).[3,43–47] Endogenous and pre-infused vWF can influence FVIII PK characteristics since vWF stabilizes FVIII heterodimeric structure, prevents FVIII plasma proteolytic degradation and inhibits FVIII hepatic cellular uptake.[3,43] Patients with von Willebrand disease (vWD) exhibit low or defective endogenous vWF, and therefore a high FVIII:C CL_{tot} has been observed in these patients.[3,43] Pre-infused vWF has been shown to have a negative correlation with FVIII:C CL_{tot}.[3,43] Blood group may influence FVIII PK characteristics secondary to coexistence of different vWF plasma levels in different blood groups.[3,43] For instance, blood group O hemophiliacs have been shown to have a high FVIII:C CL_{tot} that may be explained by presence of a low vWF plasma level. [3,43] Age and bodyweight may influence FVIII PK characteristics due to different physiological changes related to changes in these two factors.[3,43] Both age and bodyweight have been shown to have a negative correlation with FVIII:C CL_{tot}.[3,43] Different medical states may influence FVIII PK characteristics secondary to pathophysiological changes affecting FVIII. For instance, a low FVIII:C CL_{tot} has been observed postoperatively that is explained by a subsequent increase in vWF plasma level due to FVIII continuous infusion during surgery.[43,44] FVIII inhibitory antibodies can influence FVIII PK characteristics by rapid inactivation of infused FVIII therapy, thereby resulting in a high FVIII:C V_{ss} and CL_{tot}.[45]

FVIII assays play a critical role in FVIII PK variability.[46] Different FVIII assays are available, e.g., one-stage clotting, two-stage clotting and chromogenic substrate assays.[46] Different reference standards are available, e.g., international standards and product-specific standards.[46] Different assay equipment (coagulometer) and procedures (dilutions) are available.[46] Variation in FVIII assays is a major source of FVIII PK discrepancy.[46] For

instance, one-stage clotting assays have been shown generally to result in 20–50% lower plasma FVIII:C than that obtained by chromogenic substrate assays.[47]

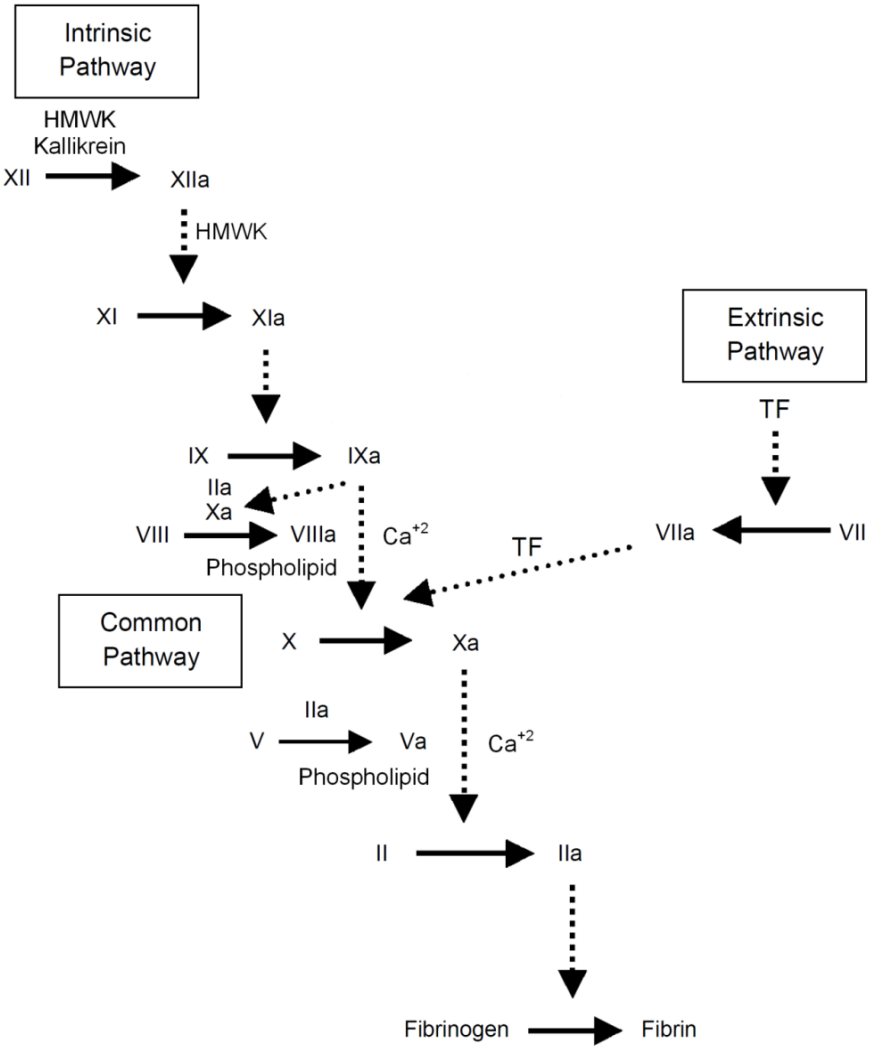
Variability in FVIII PK characteristics between pdFVIII and rFVIII products have been investigated in many crossover studies.[3,43] This variability rFVIII is generally consistent among different bioequivalence studies in hemophilia, especially under stable physiological conditions.[3,43] In general, FVIII:C V_{ss} and CL_{tot} of pdFVIII products were significantly higher than those of rFVIII products.[3,43] Variability in FVIII:C V_{ss} and CL_{tot} led to a marginal variability in FVIII:C MRT and $t_{1/2}$, i.e., a shorter FVIII:C MRT and $t_{1/2}$ of pdFVIII products compared to those of rFVIII products.[3,43] In contrast to these findings, a modest variability in FVIII:C V_{ss} and CL_{tot} with no influence on FVIII:C MRT and $t_{1/2}$ was reported in one study[42] (a lower FVIII:C V_{ss} and CL_{tot} of the pdFVIII product compared to those of the rFVIII product).[3,43] Variability in FVIII PK characteristics may be related to differences in manufacturing procedures, purity levels and pharmaceutical contents. Differences in protocols, blood sampling schedules and FVIII assays may also influence the results of different bioequivalence studies.[3,43]

1.3. BLOOD COAGULATION CASCADE MODEL:

The cascade (or waterfall) model of blood coagulation is the classical model of hemostasis.[48–50] It demonstrates a sequential activation of various blood coagulation contributors in a dependent way.[48–51] Originally, the cascade model of blood coagulation was viewed as conversion of blood coagulation contributors, initially known as blood coagulation factors, from their proenzymatic forms to their active enzymatic forms.[48,51] The original cascade model of blood coagulation was consequently adjusted to include the fact that some blood

coagulation contributors do not have an enzymatic activity, i.e., they act as blood coagulation cofactors.[48,51] The cascade model of blood coagulation includes three pathways: intrinsic, extrinsic and common (Figure 1.4).[34] The intrinsic and extrinsic pathways are independent of each other, and they end with the common pathway.[49] The endpoint of these pathways is a burst of thrombin, also known as activated factor II (FIIa), leading to formation of an insoluble fibrin mass.[,48,49,51]

Figure 1.2. Blood Coagulation Cascade Model[34]†



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1.3.1. Intrinsic Pathway:

The intrinsic pathway is initiated by blood coagulation contributors that are normally located within the vasculature.[49] High molecular weight kininogen (HMWK) converts factor XII (FXII) to activated factor XII (FXIIa) when FXII comes in contact with a negatively charged surface in blood, e.g., an activated platelet.[49] The small amount of FXIIa converts prekallikrein to kallikrein.[49] Kallikrein, along with HMWK, converts further FXII to FXIIa in an accelerated speed.[49] Both FXIIa and HMWK converts factor XI (FXI) to activated factor XI (FXIa).[49] FXIa, in turn, converts factor IX (FIX) to activated factor IX (FIXa).[49] FIXa and two later products, FXa and thrombin, converts FVIII to activated factor VIII (FVIIIa).[49] Finally, FIXa, FVIIIa and a negatively charged phospholipid (found on cell membranes) in presence of calcium ions (Ca^{2+}) form a complex known as tenase.[49] Tenase is able to convert factor X (FX) to FXa.[49] The intrinsic pathway proceeds to the common pathway with this step.[49]

1.3.2. Extrinsic Pathway:

The extrinsic pathway is initiated by blood coagulation contributors that are normally located outside the vasculature.[49] Upon an injury, tissue factor (TF) is released from the endothelium.[49] TF converts factor VII (FVII) to activated VII (FVIIa).[49] TF binds to FVIIa, forming a complex.[49] The formed TF/FVIIa complex has a similar capability of tenase, i.e., it is able to converts FX to FXa.[49] The extrinsic pathway proceeds to the common pathway with this step.[49]

1.3.3. Common Pathway:

The common pathway is initiated at the end of the intrinsic pathway and/or the extrinsic pathway.[49] Tenase from the intrinsic pathway or TF/FVIIa complex from the extrinsic pathway

convert FX to FXa.[49] Factor V (FV) is converted to activated factor V (FVa) by a later product, thrombin.[49] FXa, FVa and a negatively charged phospholipid (found on cell membranes) in presence of Ca^{2+} form a complex known as prothrombinase.[49] Prothrombinase converts prothrombin, also known as factor II (FII), to thrombin.[49] Finally, thrombin converts fibrinogen to an insoluble fibrin mass.[49]

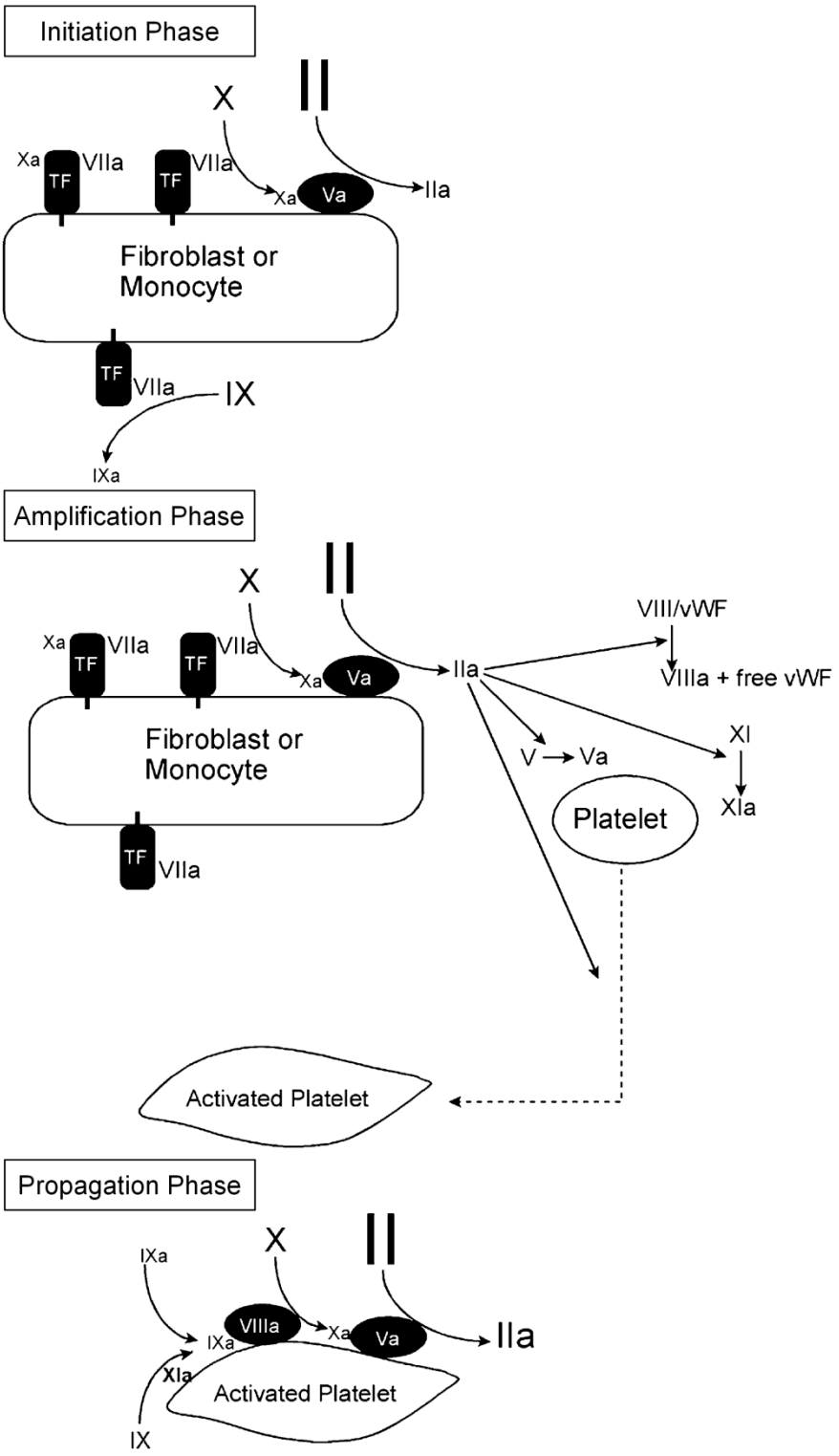
1.3.4. Blood Coagulation Cascade Model in Hemophilia A:

In hemophilia, FVIII is unavailable in the intrinsic pathway.[34] Subsequent to FVIII unavailability, FVIIIa is unavailable in the intrinsic pathway as well. Unavailability of FVIIIa results in absence of tenase at the end of the intrinsic pathway. Tenase is a critical element of the common pathway. It is required for activation of the first reaction, and therefore its absence leads to absence of the insoluble fibrin mass at the end of the common pathway. Consequently, hemophiliacs bleed spontaneously due to FVIII deficiency.

1.4. BLOOD COAGULATION CELL-BASED MODEL:

The cell-based model of blood coagulation is the modern model of hemostasis.[48–51] It exhibits the interdependent interaction between various blood coagulation contributors.[48–51] It emphasizes on the role of cells, e.g., TF-bearing cells and platelets, in the blood coagulation process.[48–51] Therefore, it adds more details to in vivo hemostasis.[48–51] The cell-based model of blood coagulation includes three phases: initiation, amplification and propagation (Figure 1.5).[48] Similar to the cascade model but with a sophisticated remodeling, the endpoint of the cell-based model of blood coagulation is a sustained thrombin generation, leading to formation of a critical, insoluble fibrin matrix between activated platelets.[48–51]

Figure 1.3. Blood Coagulation Cell-Based Model[48]†



†Modified from its original format

1.4.1. Initiation Phase:

Cells expressing TF are localized outside the vasculature, which prevents initiation of blood coagulation under normal conditions.[48,50,51] Upon injury, blood becomes exposed to TF-bearing cells, and thus begins the initiation phase.[48,50,51] FVIIa, the only blood coagulation factor circulating routinely in blood in its active form, binds to the exposed TF.[48,50,51] TF/FVIIa complex activates FIX and FX.[49,50,51] FXa activates its cofactor, FV.[49,50,51] FXa then associates with FVa, forming prothrombinase on the surface of TF-bearing cells.[48,50,51] Finally, prothrombinase cleaves prothrombin to thrombin, and the initiation phase proceeds to the amplification phase.[48,50,51]

1.4.2. Amplification Phase:

The blood coagulation process progresses to its amplification phase when the vasculature damage permits platelets and larger proteins to diffuse into the extravascular site and attach to TF-bearing cells.[48,50,51] The insignificant amount of thrombin formed in the initiation phase has several functions in the amplification phase.[48,50,51] First, it activates FV, which binds to the surface of platelets.[48,50,51] FVa is also released from platelets in its active form.[48,50,51] In addition, thrombin dissociates FVIII/vWF complex into FVIIIa, which binds to the surface of platelets, and free vWF, which mediates further adhesion and aggregation to platelets.[48,50,51] Furthermore, thrombin activates FXI on the surface of platelets.[48,50,51] Finally, thrombin activates platelets, exposing their receptors to the activated blood coagulation contributors, and the amplification phase proceeds to the propagation phase.[48,50,51]

1.4.3. Propagation Phase:

The propagation phase takes place on the surface of activated platelets.[48,50,51] FIXa, generated in the initiation phase or being generated through platelet-bound FXIa, binds to FVIIIa on the activated platelet surface forming tenase.[48,50,51] Tenase activates FX.[48,50,51] FXa binds to platelet-bound FVa, forming additional prothrombinase with adequate activity to generate additional thrombin in an accelerated speed.[48,50,51] Formation of thrombin in an accelerated speed results in dissociation of fibrinopeptide A from fibrinogen, thereby leading to formation of a critical, insoluble fibrin matrix between activated platelets.[48,50,51]

1.4.4. Blood Coagulation Cell-Based Model in Hemophilia A:

In hemophilia, the initiation phase occurs in a normal fashion. However, the amplification phase is incomplete due to FVIII deficiency. This results in deficient tenase generation in the propagation phase. Deficient tenase generation prevents self-sustaining production of FXa needed to produce a sufficient amount of prothrombinase. Lacking of prothrombinase generation, therefore, prevents further thrombin burst necessary for additional platelets activation and fibrin formation. Subsequently, patients with hemophilia experience spontaneous bleeding.

1.5. APPLICATION OF BLOOD COAGULATION CASCADE MODEL IN HEMOPHILIA A:

The cascade model of blood coagulation has been utilized to screen hemophilia and assess its severity.[52,53] It has also been utilized to quantify FVIII replacement therapy pharmacodynamic (PD) response as well as its PK characteristics.[52,53] For these purposes, both aPTT and FVIII:C have been used extensively. [52–54] Hemophilia requires assessment of blood

coagulation through the intrinsic and common pathways because FVIII, the deficient blood coagulation contributor, is a part of the intrinsic pathway, and its deficiency has a large effect on the common pathway endpoint, fibrin.[54] Therefore, absence of TF, a critical blood coagulation contributor in the extrinsic pathway, from aPTT and FVIII:C assays allow exclusive assessment of blood coagulation through the intrinsic and common pathways.[54] Both aPTT and FVIII:C are measured within the plasma matrix.[52–54]

1.5.1. Activated Partial Thromboplastin Time:

Assays of aPTT involve two phases.[52] The first phase is called the contact activation phase.[52] It includes the first reaction of the intrinsic pathway to obtain FXIIa.[52] The contact activation phase is initiated by pre-incubating the citrated plasma sample with blood coagulation activators.[52] Blood coagulation activators includes artificial contact activators, e.g., ellagic acid ,celite, kaolin or silica, that create surfaces with negative charges for the reactions to occur.[52] Phospholipids are also included as blood coagulation activators in the contact activation phase; however, they are not involved in activation of blood coagulation until the second phase.[52] The second phase is called the re-calcification phase.[52] It includes the rest of the reactions in the intrinsic and common pathways, resulting in a fibrin clot.[52] The re-calcification phase is initiated by adding calcium chloride ($CaCl_2$) that provides Ca^{+2}.[52] Ca^{+2} are involved in different reactions and necessary to reverse the anticoagulant effect of citrate in the plasma sample.[52] The formed fibrin clot density in the plasma sample is detected optically (common) or mechanically (less common) by automated methods, and aPTT is recorded.[52,54] Reference aPTT values range from 25–38 seconds (sec).[54] In hemophilia, aPTT is severely prolonged.

1.5.2. FVIII Coagulant Activity:

Like other blood coagulation factors, FVIII cannot be quantified by physiochemical assays due to its complex structure and function.[55] However, two quantification techniques have been developed utilizing two major moieties of FVIII complex: FVIII:C and factor VIII antigen (FVIII:Ag).[56] FVIII:C possesses the active biological function, and therefore it is quantified by bioassays that utilize the cascade model of blood coagulation.[55,56] These bioassays quantify FVIII:C in an indirect manner since the FVIII is a proenzyme that possesses no direct enzymatic activity.[53] Three FVIII:C bioassays are available: one-stage clotting, two-stage clotting and chromogenic substrate.[53] These assays, especially one-stage clotting assays, are common in clinical settings.[47] FVIII:Ag is inactive, and its amount is equivalent to that of FVIII:C.[56] FVIIII:Ag is quantified by immunoassays.[56] FVIII:Ag immunoassays are irrelevant to the cascade model of blood coagulation and rarely being used in clinical settings.

Principles and techniques of one-clotting assays are similar to aPTT assays.[47,53] One-stage clotting assays quantify FVIII ability to shorten aPTT in one step.[47,53] One-stage clotting assays utilize the intrinsic and common pathways to quantify aPTT as described before. However, they assume that FVIII, the deficient coagulation contributor in hemophilia, is rate-limiting in the plasma sample with all blood coagulation contributors available at their saturating levels.[53] One-stage clotting assays also assume that there is a straight mathematical relationship between aPTT and FVIII:C, and therefore FVIII:C can be obtained by matching the assay aPTT to a reference using a standard plasma FVIII:C-aPTT profile on a logarithmic scale.[57] A major limitation of the one-stage clotting assays is feedback activation of FVIII in the intrinsic pathway by thrombin formed in the common pathway.[53] One-stage clotting assays are the most commonly used in clinical settings.[47]

Principles and techniques of two-stage clotting assays are similar to that of one-stage clotting assays with few modifications, though.[47,53,57] In two isolated steps, two-stage clotting assays quantify FVIII ability to potentiate FX activation with subsequent prothrombinase formation by measuring the rate of fibrin formation.[47,53,57] The first step includes all the reactions in the intrinsic pathway and the first few reactions in the common pathway necessary to form FXa and prothrombinase.[47,53,57] The first step is initiated by pre-incubating the citrated plasma sample with phospholipids, $CaCl_2$, FV, FIX, FX, FXI, FXII and aluminum hydroxide ($Al[OH]_3$).[53,57] FV, FIX, FX, FXI and FXII are added to guarantee that FVIII, the deficient blood coagulation contributor, is rate-limiting for FXa and prothrombinase formation in the plasma sample, and therefore the amount of FXa and prothombinase are proportional to the amount of FVIII in the plasma sample. $Al(OH)_3$ adsorbs prothrombin from the plasma sample, which prevents progression of blood coagulation beyond FXa and prothombinase formation in the first step, thereby preventing feedback activation of FVIII in the intrinsic pathway by thrombin formed in the common pathway.[57] The second step includes the rest of reaction in the common pathway after FXa and prothrombinase formation and until fibrin formation.[47,53] It is initiated by adding prothrombin and fibrinogen to the plasma sample.[57] Fibrin formation is detected optically or mechanically by automated methods. Fibrin formation time is recorded accordingly.[57] As for one-stage clotting assays, two-stage clotting assays assume that there is a direct mathematical relationship between fibrin formation time and FVIII:C, and therefore FVIII:C can be obtained by matching the assay fibrin formation time to a reference using a standard plasma FVIII:C-fibrin formation time profile on a logarithmic scale.[57] Due to the complexity with automation of two-stage clotting assays, they are not commonly used.[58]

Principles and techniques of chromogenic substrate assays involves two steps as described before for the two-stage clotting assays with, however, an adjusted detection method.[47,53,57] The detection method includes addition of a chromogenic substrate for FXa in the second step.[53] FXa cleaves the chromogenic substrate, and the color reaction (absorbance) is measured spectrophotometrically.[47,53,57] The color intensity is proportional to the amount of FXa, which is proportional to the amount of FVIII in the plasma sample (assuming that FVIII is rate-limiting for FXa formation in the first step), and FVIII:C can be calculated accordingly.[47,53,57] Whenever chromogenic substrate assays are available, they are favored over two-stage clotting assays due their less complex automation.

1.6. APPLICATION OF BLOOD COAGULATION CELL-BASED MODEL IN HEMOPHILIA A:

The cell-based model of blood coagulation has been utilized to develop different assays that provide biomarkers assessing global hemostasis.[59,60] Under ex vivo environment, their principles and techniques mimic in vivo blood coagulation.[59,60] Therefore, global hemostasis biomarkers can potentially assess the hemostatic state and quantify FVIII replacement therapy PD response in hemophilia. Global hemostasis biomarkers are classified into two classes: platelet function and blood viscoelasticity.[59,60] Platelet function is assessed using Hemostasis Analysis System (HAS) that provides platelet contractile force (PCF), clot elastic modulus (CEM) and force onset time (FOT).[59] Blood viscoelasticity can be assessed by Thromboelastography (TEG) that provides reaction-time (R), kinetics-time (K) and maximum amplitude (MA).[60] Global hemostasis biomarkers have been validated in different thrombotic and non-thrombotic disorders, including hemophilia.[59–69] Platelet function and blood viscoelastic biomarkers are measured within the whole blood matrix.[59,60]

1.6.1. Platelet Function Biomarkers:

Platelets play a critical role in the cell-based model of blood coagulation.[48–51] Fibrin polymers connect activated platelets at the end of the blood coagulation process.[48–51] Therefore, assessment of platelet function by HAS may be critical in hemophilia, reflecting the entire blood coagulation contributors, including FVIII. Blood coagulation in HAS is initiated by re-calcification of a citrated whole blood sample in a thermostated analyzer cup, and platelet function is assessed over 20 minutes (min).[61] The re-calcification provides Ca^{+2} that are involved in different blood coagulation reactions and necessary to reverse the anticoagulant effect of citrate in the whole blood sample.[52] As HAS is running, an upper plate centered above the analyzer cup containing the whole blood sample is lowered.[61] As the clot develops, the clot adheres to the exposed surface of the analyzer cup and the upper plate.[61] As fibrin is formed between platelets, platelets pull the fibrin polymers to the center generating force to the clot that is attached to the surface of the analyzer cup and the upper plate.[61] A transducer attached to the upper plate generates electrical signals (measured as kilodynes [kdynes]) proportional to the platelets contractions, which are recorded continuously throughout the assay time.[61] These records are known as PCF.[61]

A descending force with a specific magnitude is also applied to the upper plate.[61] The extent of the descending displacement resulted from the force is determined.[61] The ratios of the descending force to the descending displacement (measured in kilodynes per centimeter square [kdynes cm^{-2}]) are computed throughout the assay time.[61] These ratios indicate the elasticity of the formed clot between the analyzer cup and the upper plate.[61] These ratios are known as CEM.[61]

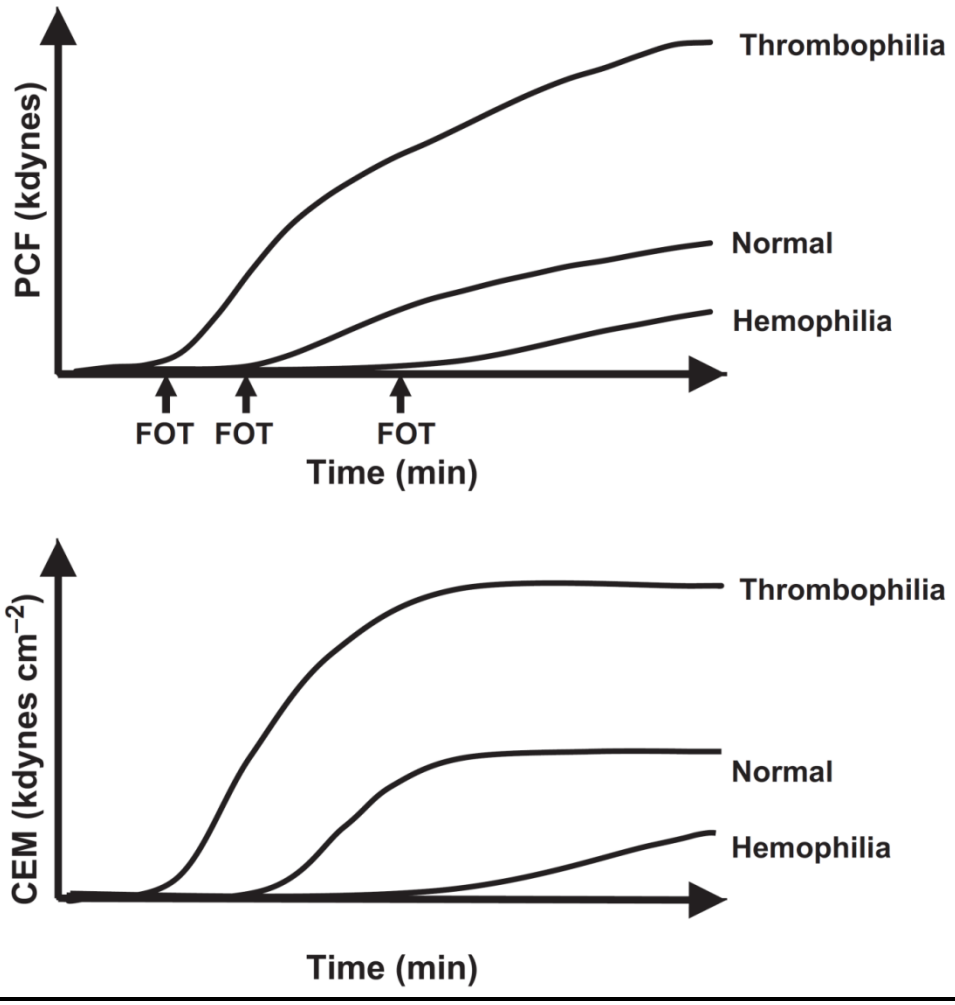
Re-calcification of the whole blood sample results in initial thrombin generation since blood coagulation is initiated immediately.[61] The initial amount of thrombin generated in the whole blood sample between the analyzer and the upper plate is essential for initial platelets contractions, which is indicated by initially recorded PCF.[61] Therefore, the lag time before initially recorded PCF indicates the time required to generate thrombin.[61] This lag time is known as FOT.[61] FOT is determined from the PCF-time profile by observing the first spike in PCF[61]

Platelet function biomarkers, including PCF, CEM and FOT, quantify in vivo blood coagulation.[59,61] PCF is the force generated by platelets throughout clot retraction.[59,62–64] PCF is sensitive to platelets (number and metabolic status), thrombin, antithrombins and glycoprotein IIb/IIIa (GPIIb/IIIa) platelet receptor status.[59,62–67] CEM reflects clot structural firmness.[59,62–64] CEM is sensitive to platelets (number and force), thrombin generation rate, fibrinogen concentration, red blood cell (RBC) flexibility and changes in clot structure.[59,62–66] FOT, also referred to as thrombin generation time (TGT), is the time in which thrombin is produced in whole blood.[59,61–64] FOT reflects clotting initiation.[59,61–64] FOT is estimated from PCF kinetics.[59,61–64] As PCF is thrombin-dependent, the initial spike in PCF, indicated by FOT, is a measure of thrombin generation.[59,61–64] FOT is sensitive to blood coagulation factor deficiencies and inhibitors, as well as antithrombins.[59,62] Reference ranges for platelets function biomarkers are shown in Table 1.11.[63] Significantly low PCF, low CEM and prolonged FOT are related to increased risk of bleeding, e.g., hemophilia, while significantly high PCF, high CEM and shortened FOT are related to increased risk of thrombosis, e.g., coronary artery disease (CAD).[59] Figure 1.4 shows platelet function biomarkers in different conditions.[63]

Table 1.11. Reference Ranges for Platelet Function Biomarkers[63]

Biomarker	Reference Range
PCF (kdynes)	4.8–9.5
CEM (kdynes cm^{-2})	14.0–35.0
FOT (min)	3.0–8.0

Figure 1.4. Platelet Function Biomarkers in Different Conditions[63]

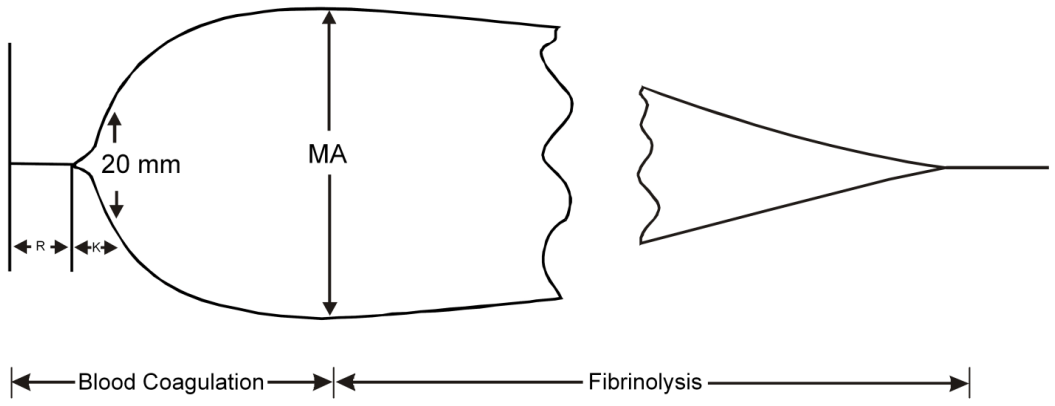


1.6.2. Blood Viscoelastic Biomarkers:

Fibrin polymers formed at the end of the cell-based model of blood coagulation causes changes in blood viscoelasticity.[60] Consequently, assessment of blood viscoelasticity by TEG

may be vital in hemophilia, catching changes in the blood coagulation contributors, including FVIII. Blood coagulation in TEG is initiated by transferring a citrated whole blood sample pre-mixed with blood coagulation activators, e.g., phospholipids and an artificial contact activator (kaolin), to a thermostated analyzer cup containing $CaCl_2$, and blood viscoelasticity is assessed over time.[60,70] The artificial contact activator creates surfaces with negative charges for the blood coagulation reactions to occur.[52] Phospholipids are involved in many reactions.[48–51] $CaCl_2$ is a source of Ca^{+2}, which are involved in different reactions and necessary to reverse the anticoagulant effect of citrate in the whole blood sample.[52] As TEG is running, the analyzer cup oscillates backward and forward at a constant speed.[60,71] A stationary pin connected to a torsion wire is suspended in the analyzer cup containing the whole blood sample.[60,71] As the clot develops, the resistance changes and the analyzer cup motions are detected over time by the stationary pin.[60,71,72] The pin transmits the detected motions to a computer that graphically represents clot development, and blood viscoelastic biomarkers are calculated (Figure 1.5).[72,73,74]

Figure 1.5. TEG Tracing with Blood Viscoelastic Biomarkers[74]†



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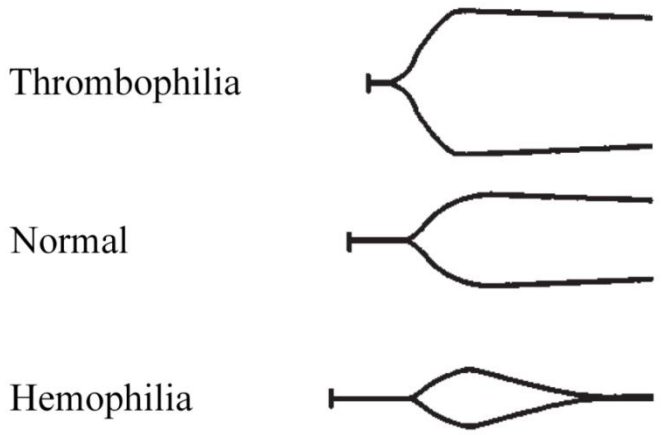
Blood viscoelasticity assessed by TEG includes two phase: blood coagulation and fibrinolysis.[74] Routinely reported blood viscoelastic biomarkers include R, K and MA from the blood coagulation phase.[71] R is the lag time before initial clot formation.[63] R is actually the time between transferring the whole blood sample to the analyzer cup and an initial signal of 2 millimeters (mm) amplitude.[63] Therefore, R indicates the rate of fibrin production and cross-linking.[63] K is the time between initial clot formation to integrated clot development.[60] K is actually the time the initial signal of 2 mm amplitude to a signal of 20 mm amplitude.[63] Therefore, K expresses the dynamic of clot development and propagation.[63] MA quantifies the maximum integrated fibrin clot (strength and stability).[60,63] MA is actually the maximum height of the TEG trace.[60] MA is largely dependent on plasma fibrinogen content and platelet count.[63] Therefore, MA is also dependent on fibrin (content and structure) and platelet concentration.[63] Reference ranges for blood viscoelastic biomarkers are shown in Table 1.12.[63] Significantly prolonged R, prolonged K and decreased MA are related to increased risk of bleeding, e.g., hemophilia, while significantly shortened R, shortened K and increased MA are related to increased risk of thrombosis, e.g., deep vein thrombosis (DVT).[60,71] Figure 1.6 shows TEG tracing in different conditions.[71]

Table 1.12. Reference Ranges for Blood Viscoelastic Biomarkers[63]

Biomarker	Reference Range
R (min)	3.0–8.0
K (min)	1.0–3.0
MA (mm)	51.0–69

Figure 1.6. TEG Tracing in Different Conditions[71]†



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1.7. PREVIOUS FACTOR VIII PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS:

1.7.1. Previous Factor VIII Pharmacokinetic Modeling:

A PK model describes the resulting plasma level over time for a dug, and therefore different PK parameters can be established.[75] FVIII cannot be quantified by physiochemical assays due to its complex structure and function.[55] Therefore, it is quantified in clinical settings by bioassays utilizing its coagulant activity (FVIII:C).[47,55] FVIII PK models can be established by non-compartmental analysis (NCA) as well as by compartmental analysis (CA).[46] FVIII NCA, also known as model-independent or free analysis, does not deal with FVIII PD response.[46] It is also less influenced by best-fitting procedures (tedious processes subjected to errors).[46] Therefore, it is popular among agencies concerned with FVIII bioequivalence.[46] It can also be used as a reference for FVIII CA. FVIII CA deals with FVIII PD response.[46] Therefore, it is common among clinical PK studies.[46] However, it is influenced by best-fitting

procedures that are subjected to errors (based on the blood sampling schedule and FVIII assay sensitivity/specificity).[46,76]

FVIII CA includes the one-compartment body model as well as the two-compartment body model.[76] The choice of FVIII CA depends on the terminal β phase (disposition part) of the plasma FVIII:C-time profile.[76] The one-compartment body model is used upon observing a monophase in the terminal β phase, which indicates distribution of FVIII in only one compartment (Figure 1.7).[76] The two-compartment body model is used upon observing a biphase in the terminal β phase, which indicates distribution of FVIII in two compartments (Figure 1.8).[76] Obtaining a monophase or a biphase in the terminal β phase is influenced by the blood sampling schedule and FVIII assay sensitivity/specificity.[46,76] A monophse in the terminal β phase has been observed by majority of FVIII CA studies, suggesting the one-compartment body model for FVIII replacement therapy.[76]

Figure 1.7. FVIII One-Compartment Body Model on a Logarithmic Scale

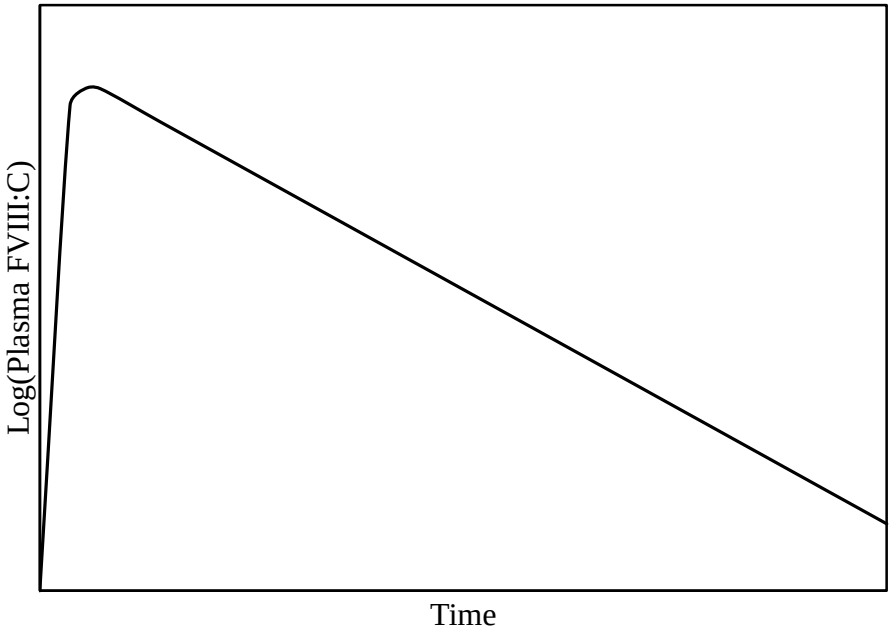
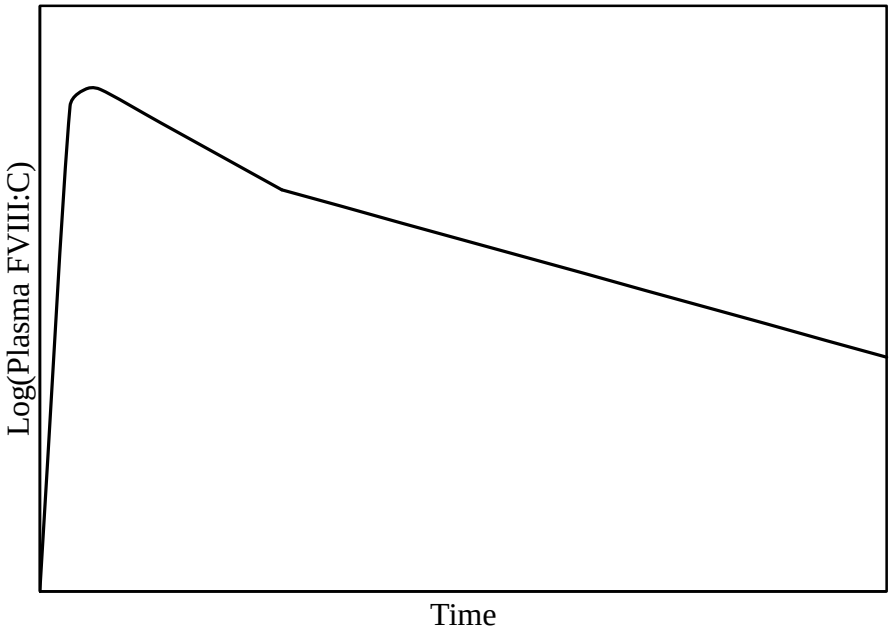


Figure 1.8. FVIII Two-Compartment Body Model on a Logarithmic Scale



1.7.2. Previous Factor VIII Pharmacokinetic/Pharmacodynamic Modeling:

A PK/PD model describes the resulting PD response over time for a drug and mathematically relates that PD response to the drug plasma level.[75] Consequently, it allows establishment of different PD parameters. Current, as well as early, therapeutic drug monitoring (TDM) guidelines for FVIII replacement therapy relies heavily on FVIII PK models because hemophilia is actually FVIII deficiency, and its treatment revolves around replacing the deficient blood coagulation contributor, FVIII.[77–82] Consequently, appropriate FVIII PK/PD models are lacking. FVIII PK models are developed in clinical settings by quantification of FVIII plasma levels using its plasma coagulant activity (plasma FVIII:C), which is quantified by appropriate bioassays (one-stage clotting, two-stage clotting or chromogenic substrate).[47,52–55,57,58] All current FVIII bioassays include an appropriate PK/PD model to quantify plasma FVIII:C.[47,52–55,57,58] One-stage clotting assays include aPTT as a PD response.[47,52–54,57,58] Two-stage clotting assays include fibrin formation time as a PD response.[47,53,57,58] Chromogenic substrate assays include FXa ability to cleave a chromogenic substrate with a subsequent color absorbance as a PD response.[47,53,57,58] All FVIII bioassays assume linearity in FVIII PD response over time (Figures 1.9 and 1.10).[53,57] Therefore, FVIII PK/PD models are centered on the linear model.

Figure 1.9. FVIII PK/PD Linear Model Time Dependent Profile

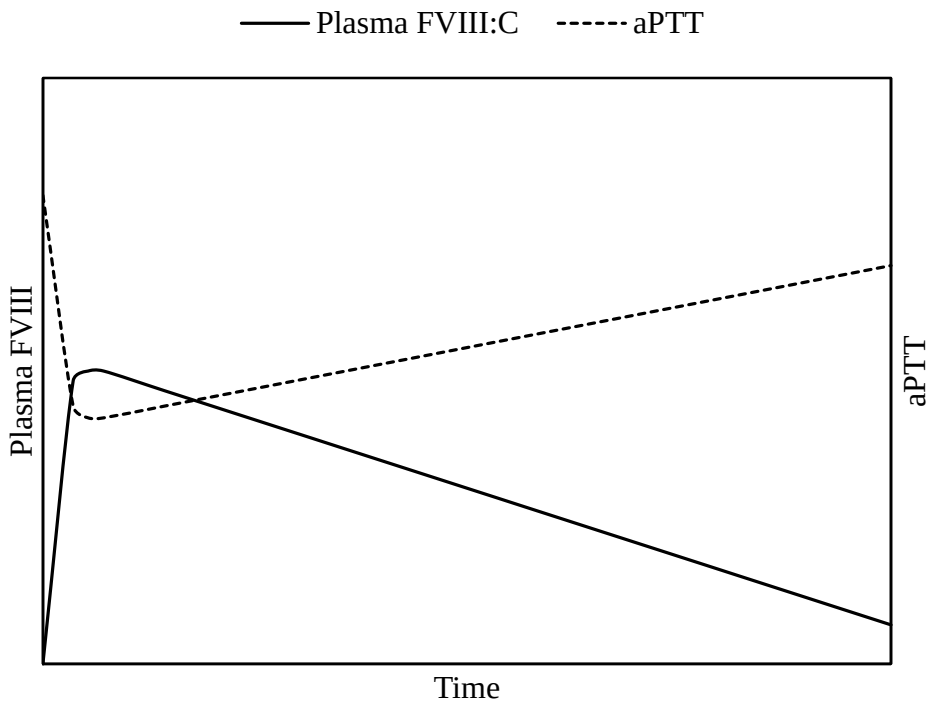
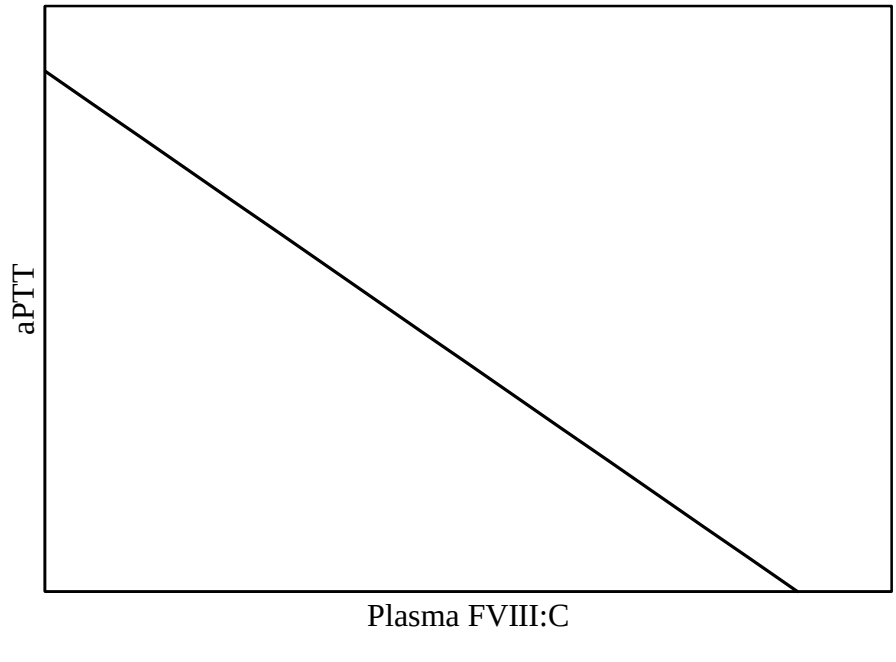


Figure 1.10. FVIII PK/PD Linear Model Plasma FVIII:C Dependent Profile



CHAPTER 2. THEORETICAL AND CONCEPTUAL APPROACH

2.1. LITERATURE REVIEW:

2.1.1. Factor VIII Prophylaxis:

Among FVIII replacement therapy strategies, FVIII prophylaxis is becoming an accepted practice to reduce the prevalence of bleeding in severe hemophilia.[83] The current clinical practice of FVIII prophylaxis revolves around converting patients with severe hemophilia phenotype (defined as plasma FVIII:C <1 IU dL^{-1}) to moderate hemophilia phenotype (defined as plasma FVIII:C from 1–5 IU dL^{-1}).[18,78–84] Such a procedure is hypothesized to reduce bleeding episodes in severe hemophiliacs, and subsequently preventing unfavorable outcomes, e.g., joint deterioration and muscle impairment.[18,78–84] Therefore, FVIII prophylaxis in severe hemophilia remains controversial.[85–87] However, several public health organizations and societies, e.g., World Health Organization (WHO), the World Federation of Hemophilia (WFH) and the Medical and Scientific Advisory Council (MASAC) of the United States National Hemophilia Foundation, consider its use in severe hemophiliacs with frequent bleeding episodes as a standard care.[88]

2.1.2. Bleeding Variability in Hemophilia A:

A wide inter-individual variation in bleeding tendency, either frequent or infrequent, is observed in hemophilia (Table 2.1).[89–93] Within this paradigm, many hemophiliacs with trough plasma FVIII:C ≥1 IU dL^{-1} (moderate or mild hemophilia phenotype) may experience a frequent breakthrough bleeding.[89–91] Conversely, several hemophiliacs with plasma FVIII:C <1 IU dL^{-1} (severe hemophilia phenotype) may exhibit an infrequent bleeding.[89,92,93] Therefore, simply monitoring plasma FVIII:C in hemophiliacs to make clinical decisions regarding bleeding risk is flawed. A potential explanation is that current TDM standards, including plasma FVIII:C and aPTT, are measured within the plasma matrix, which reflects the classic model of blood coagulation (the cascade model [a sequential activation of various blood coagulation contributors in a dependent way]).[48–54] Plasma FVIII:C and aPTT demonstrate neither the dynamic role of platelets nor the blood viscoelastic changes in hemophilia. Therefore, monitoring the hemostatic process in hemophilia using global hemostasis (platelet function and blood viscoelastic) biomarkers within the whole blood matrix may be more beneficial, especially that they reflect the modern model of blood coagulation (the cell-based model [an interdependent interaction between various blood coagulation contributors]).[48–51,59,60] Global hemostasis biomarkers introduce the role of platelets and the blood viscoelastic changes in hemophilia.[59,60]

Table 2.1. Representative Studies Showing Bleeding Variability in Hemophilia

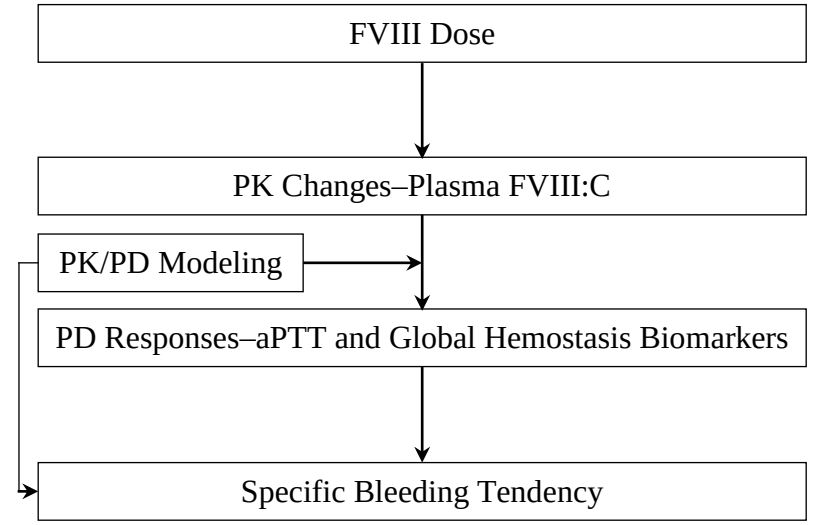
Study	Year	Design	n	Key Results
Ahnström et al.[89]	2004	Retrospective cohort with prospective PK analysis	51	• Frequent joint bleeding with plasma FVIII:C ≥1 IU dL^{-1} • Infrequent joint bleeding with plasma FVIII:C <1 IU dL^{-1}
van den Berg et al.[90]	2002	Retrospective cohort	70	• No change in joint bleeding with plasma FVIII:C ≥1 IU dL^{-1}
Carlsson et al[91]	1997	Randomized crossover PK analysis	21	• Frequent joint bleeding with plasma FVIII:C ≥1 IU dL^{-1}
Petrini[92]	2001	Retrospective cohort	43	• Infrequent joint bleeding despite plasma FVIII:C
Ghosh et al.[93]	2001	Case-series	11	• Mild clinical presentations with plasma FVIII:C <1 IU dL^{-1}

2.2. FACTOR VIII PHARMACOKINETIC/PHARMACODYNAMIC MODELING RATIONALE:

In general, the corresponding plasma levels to dosing regimens of different drugs are best interpreted in the context of their biological responses.[94] Therefore, considering their PK and PD aspects is a scientific approach used to modify, optimize and individualize their dosing regimens.[94] In severe hemophilia, this scientific approach is applicable (Figure 2.1). An infusion of a FVIII prophylactic dose causes changes in plasma FVIII:C, i.e., PK changes, throughout its prophylactic interval. Subsequent changes in plasma FVIII:C exert responses on aPTT and global hemostasis (platelet function and blood viscoelastic) biomarkers, i.e., PD responses, throughout the FVIII prophylactic interval. The PK changes and the PD responses contribute to specific bleeding tendency, either frequent or infrequent. Therefore, relating the PK and PD aspects of FVIII prophylactic dosing by appropriate PK/PD modeling may allow assessment of

specific bleeding tendency in severe hemophilia. However, how plasma FVIII:C of FVIII prophylactic dosing relates to global hemostasis biomarkers in severe hemophilia represents a critical gap knowledge. Accordingly, appropriate PK/PD modeling using FVIII:C and global hemostasis biomarkers may enable to optimize and individualize FVIII prophylactic dosing for less breakthrough bleeding in severe hemophilia.

Figure 2.1. FVIII PK/PD Modeling Rationale

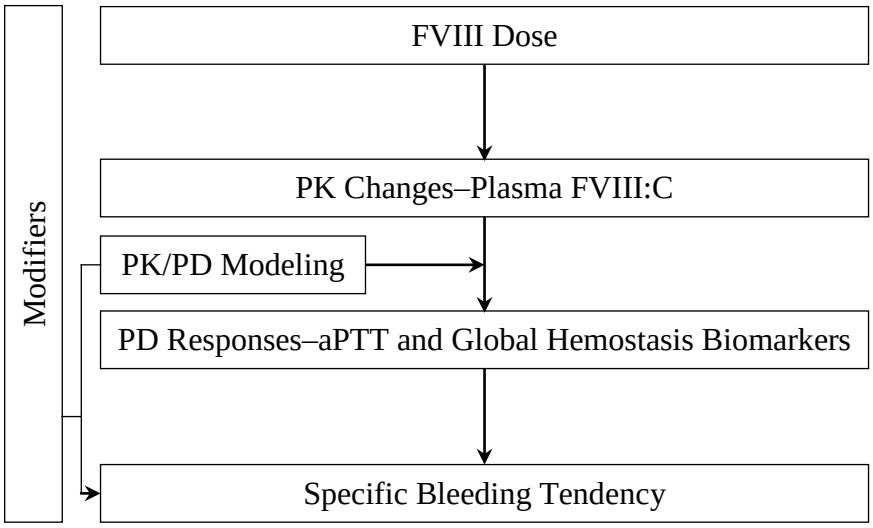


2.3. ROLE OF MODIFIERS ON BLEEDING TENDENCY:

Several factors may modify bleeding tendency in hemophilia (Figure 2.2). Fibrinogen and platelets are important blood coagulation contributors.[48–51] Therefore, plasma fibrinogen content (measured in milligram per deciliter [mg L^{-1}]) and platelet count (measured per liter [L^{-1}]) may modify specific bleeding tendency in hemophilia. Age, bodyweight and ethnicity are associated with multi-prothrombotic factors, e.g., pathophysiological changes, underlying diseases and gene expressions.[95–97] In addition, age and bodyweight may influence FVIII PK characteristics.[3,43] Therefore, age, bodyweight and ethnicity may modify specific bleeding

tendency in hemophilia. Different FVIII prophylactic regimens, e.g., two times per week or three times per week, may affect FVIII PK characteristics and its PD responses, and therefore specific bleeding tendency may be modified in hemophilia. Exploring clinically relevant modifiers of bleeding tendency may allow accurate assessment of specific bleeding tendency by appropriate PK/PD modeling for more optimal, individualized FVIII prophylactic dosing in severe hemophilia.

Figure 2.2. Role of Modifiers on Bleeding Tendency in Hemophilia



2.4. OBJECTIVE AND CENTRAL HYPOTHESIS:

The **longstanding goal** of this study was to establish optimal, individualized FVIII prophylactic dosing for less breakthrough bleeding in severe hemophilia. In order to accomplish this goal, we postulated employing global hemostasis (platelet function and blood viscoelastic) biomarkers, along with plasma FVIII:C, to optimize and individualize FVIII prophylactic dosing in severe hemophilia. Accordingly, the **objective** of this study, which was the initial stage toward accomplishing its longstanding goal, was to conduct appropriate PK/PD modeling using plasma

FVIII:C and global hemostasis (platelet function and blood viscoelastic) biomarkers in severe hemophilia. Therefore, the **central hypothesis** of this study was that following FVIII prophylactic dosing, specific bleeding tendency may be assessed by identifying how plasma FVIII:C corresponds to global hemostasis (platelet function and blood viscoelastic) biomarkers in an individual with severe hemophilia.

2.5. SPECIFIC AIMS:

The concepts and techniques in this study advanced the pharmacotherapy of hemophilia by challenging the current TDM standards for FVIII prophylaxis (plasma FVIII:C and aPTT) using novel global hemostasis (platelet function and blood viscoelastic) biomarkers. Briefly, the following specific aims (Figure 2.3) were conducted to achieve the objective of this study and evaluate its central hypothesis in severe hemophilia phenotype (plasma FVIII:C <1 IU dL^{-1}) with different bleeding tendency (frequent versus infrequent):

1.A. determine FVIII PK profiles over 48-h following FVIII prophylaxis dosing using plasma FVIII:C;

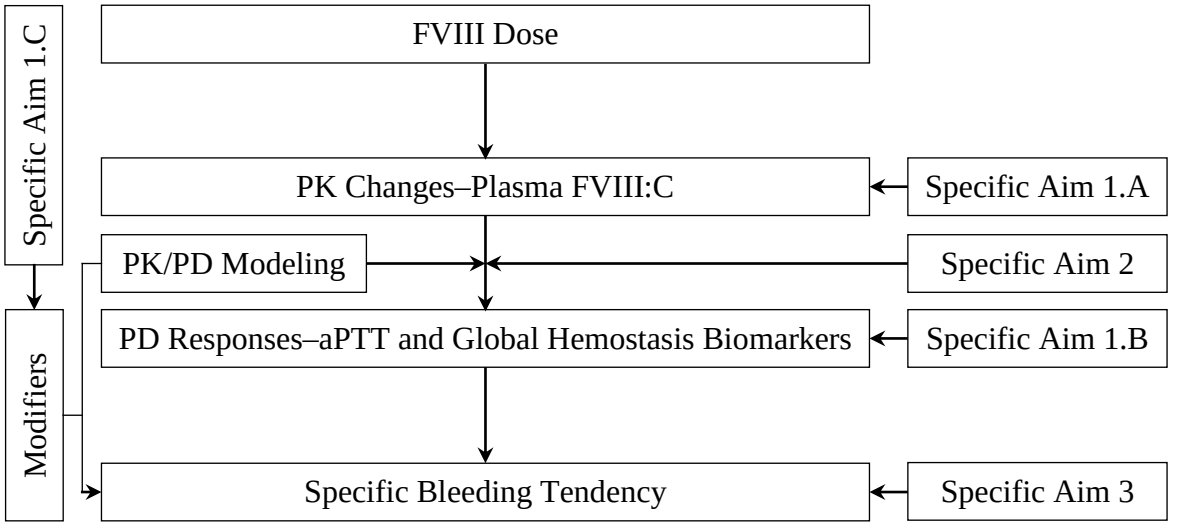
1.B. determine FVIII PD profiles over 48-h following FVIII prophylaxis dosing using aPTT and global hemostasis biomarkers (platelet function [PCF, CEM and FOT] and blood viscoelasticity [R, K and MA]);

1.C. explore clinically relevant modifiers of bleeding tendency, including plasma fibrinogen content, platelet count, age, bodyweight, ethnicity and FVIII prophylactic regimen;

2. develop appropriate PK/PD models using FVIII PK and PD profiles determined over 48-h following FVIII prophylaxis dosing; and

3. assess FVIII PK/PD models and clinically relevant modifiers of bleeding tendency quantitatively.

Figure 2.3. Fitting the Specific Aims to the Study Rationale



2.6. SIGNIFICANCE:

Patients with severe hemophilia are predisposed to recurrent bleeding episodes.[18] Such bleeding episodes result in progressive joint deterioration and muscle impairment.[18] These kind of disabilities subsequently lead to poor quality of life and economic burden due to extensive utilization of healthcare resources.[11,18,98] The significance of this study is centered on its potential to provide a solid foundation for clinicians to assess bleeding tendency in severe hemophiliacs receiving FVIII prophylaxis using novel global hemostasis (platelet function and blood viscoelastic) biomarkers. For instance, instead of following plasma FVIII:C after prescribing a FVIII prophylactic dose and hoping to prevent bleeding episodes in patients with severe hemophilia, clinicians will have a chance to monitor more meaningful global hemostasis biomarkers throughout the FVIII prophylactic interval. Clinicians can then adjust FVIII

prophylactic dosing in more effective patient-specific need by observing normalized global hemostasis biomarkers throughout the FVIII prophylactic interval. Therefore, more optimal, individualized FVIII prophylactic dosing based on global hemostasis biomarkers in severe hemophilia may provide more cost-effective therapy by reducing bleeding episodes, hospitalizations and utilization of expensive FVIII products. Pilot data from this study will also provide a basis for future clinical trials of optimizing and individualizing FVIII prophylaxis in severe hemophilia.

3.8. PRELIMINARY DATA:

During a previous clinical study, we have obtained blood samples from patients with hemophilia at least 48 h following FVIII prophylaxis to asses plasma FVIII:C and the corresponding platelet function biomarkers, including PCF, CEM and FOT (Table 2.2).[64] Current dogma suggests that the concept of FVIII prophylaxis is to convert patients with severe hemophilia phenotype (defined as plasma FVIII:C <1 IU dL^{-1}) to moderate hemophilia phenotype (defined as plasma FVIII:C from 1–5 IU dL^{-1}).[18,78–84] However, we were able to show that there is a disconnect between simply following plasma FVIII:C and assuming protective hemostatic PD responses on platelet function. Although the patients marked with obelisk all have plasma FVIII:C ≥1 IU dL^{-1}, they still have severely abnormal platelet function biomarkers (low PCF, low CEM and prolonged FOT). In fact, blood from the majority of these patients did not clot within 20 min, or at best it made a semi-formed liquid clot. This suggests that simply maintaining a trough plasma FVIII:C ≥1 IU dL^{-1} may not assure adequate platelet function. These data may also help explain the variability in bleeding tendency in patients with hemophilia who achieve a trough plasma FVIII:C ≥1 IU dL^{-1}.

Table 2.2. Preliminary Data[64]

ID	Plasma FVIII:C (IU dL^{-1})	PCF (kdynes)	CEM (kdynes cm^{-2})	FOT (min)
Patient 1	<1.0	0.40	0.00	≥20
Patient 2	<1.0	0.38	0.00	≥20
Patient 3	<1.0	0.22	0.00	≥20
Patient 4	<1.0	0.27	0.00	≥20
Patient 5	<1.0	0.37	0.00	≥20
Patient 6	<1.0	0.69	0.00	≥20
Patient 7†	5.0	0.80	0.00	≥20
Patient 8†	6.0	0.49	0.00	≥20
Patient 9†	4.0	0.56	0.00	≥20
Patient 10†	5.0	0.84	0.00	≥20
Patient 11†	6.0	2.47	5.91	15.0
Patient 12†	14.0	2.10	6.46	15.0
Patient 13†	1.0	0.44	0.00	≥20
Patient 14†	1.0	1.00	0.00	≥20
Patient 15	<1.0	0.60	0.00	≥20
Patient 16	<1.0	0.00	0.00	≥20
Patient 17†	1.0	0.41	0.00	≥20
Patient 18	<1.0	0.49	0.00	≥20
Patient 19†	4.0	1.45	6.39	15.0
Patient 20†	1.0	0.44	0.00	≥20
Patient 21	<1.0	0.56	0.00	≥20

†Patients with a trough plasma FVIII:C ≥1 IU dL^{-1}

CHAPTER 3. METHODS

3.1. STUDY DESIGN:

This was a prospective, open-label, single-dose, PK/PD pilot study. It established the PK/PD relationship of rFVIII prophylactic dosing using plasma FVIII:C (PK changes) and global hemostasis biomarkers (PD responses) over a 48-h prophylactic interval in severe hemophilia (plasma FVIII:C <1 IU dL^{-1}). Global hemostasis biomarkers included PCF, CEM and FOT for assessment of platelet function and R, K and MA for assessment of blood viscoelasticity. This study also included aPTT as a PD response. This study assessed variability in bleeding tendency using appropriate PK/PD models. This study also explored the role of clinically relevant modifiers of bleeding tendency, including plasma fibrinogen content, platelet count, age, bodyweight, ethnicity and rFVIII prophylactic regimen. This study was awarded by the A.D. Williams Research Grant Committee.

3.2. STUDY POPULATION:

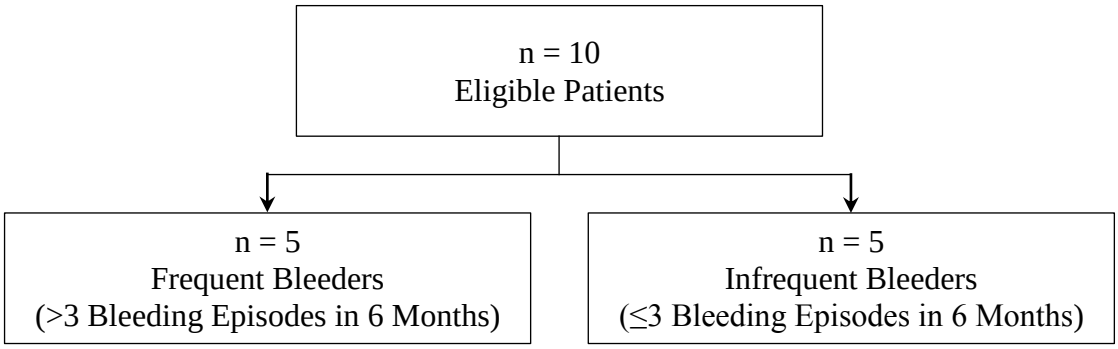
Prior to patient enrollment, the Virginia Commonwealth University (VCU) Institutional Review Board (IRB) approved this study. This study was conducted in compliance with the Declaration of Helsinki and the Health Insurance Portability and Accountability Act (HIPAA). Patients with eligibility criteria shown in Table 3.1 were identified at the Central Virginia Center for Coagulation Disorders (CVCCD), a registered HTC on the VCU Medical Center. Eligible patients were classified according to their historical bleeding tendency into two groups: frequent

and infrequent bleeders (Figure 3.1). Frequent bleeding tendency was defined as having >3 bleeding episodes in major joints or muscles during the 6 months prior to study enrollment. Infrequent bleeding tendency was defined as having ≤3 bleeding episodes in major joints or muscles during the 6 months prior to study enrollment. This study was intended to enroll 10 patients (5 patients per study group). Since this was a pilot study that aimed to acquire preliminary data to develop additional clinical trials with adequate power, the sample size (n = 10) was based on the budgetary constraints of the A.D. Williams Grant.

Table 3.1. Eligibility Criteria

Inclusion Criteria	Exclusion Criteria
• Severe hemophilia (plasma FVIII:C <1 IU dL^{-1})	• Prior FVIII dosing within 72 h of study procedures
• Receiving rFVIII prophylaxis	• FVIII inhibitory antibodies
• ≥18 years of age	• Active bleeding
• Provision of written informed consent	• Medical or family history of thrombosis

Figure 3.1. Sample Size and Study Group Classification



3.3. STUDY PROCEDURES AND DATA COLLECTION PLAN:

3.3.1. Screening Visit:

Screening visits were conducted in the CVCCD. Following the provision of written informed consent (Appendix 1), patients were screened for the eligibility criteria. Anonymous identification (ID) numbers were assigned to maintain confidentiality of patients. Demographics, FVIII deficiency information, rFVIII prophylaxis data, medical history (including bleeding tendency), family thrombotic history and concomitant medications were recorded in the screening visit form (Appendix 2). Patients received a physical examination to rule out active bleeding. A peripheral intravenous (IV) catheter was inserted for blood collection. Blood was collected in two Vacutainer 4.5-millimeters (mL) glass tubes containing 0.105 molar (M) trisodium citrate (BD, Franklin Lakes, NJ). Blood samples were processed to determine different study variables (Table 3.2). All study documents were stored in a secure, locked filing cabinet after abstraction of their data in appropriate electronic files, which were maintained in a secure, password protected computer.

3.3.2. Inpatient Visit:

Eligible patients were admitted for inpatients visits in the VCU General Clinical Research Center (GCRC). Information of the inpatient visits were recorded in the inpatient visit form (Appendix 3). Patients received a brief physical examination to rule out active bleeding. A peripheral IV catheter was inserted for rFVIII prophylactic dose infusion and blood collection. Patients were infused with their routine rFVIII prophylactic dose over 5 min. Blood was collected at baseline and at 0.5-, 1-, 2-, 4-, 8- and 12-h post-rFVIII prophylactic dose infusion in two Vacutainer 4.5-mL glass tubes containing 0.105 M trisodium citrate (BD, Franklin Lakes,

NJ). Blood samples were processed to determine different study variables (Table 3.2). Chronic medications were allowed throughout the study. Patients received a physical examination before the discharge from their inpatient visits to rule out any potential risk.

3.3.3. Outpatient Visit:

Patients returned to the CVCCD for outpatient visits at 24-h post-rFVIII prophylactic dose infusion. Information of the outpatient visits were recorded in the outpatient visit form (Appendix 4). Patients received a brief physical examination to rule out active bleeding. A peripheral IV catheter was inserted for blood collection. Blood was collected in two Vacutainer 4.5-mL glass tubes containing 0.105 M trisodium citrate (BD, Franklin Lakes, NJ). Blood samples were processed to determine different study variables (Table 3.2). Patients received a physical examination before the release from their outpatient visits to rule out any potential risk.

Table 3.2. Blood Sampling Schedule

		Time Post-rFVIII Dose Infusion (h)								
Variable	**Screening**	**0**	**½**	**1**	**2**	**4**	**8**	**12**	**24**	**48**
Plasma FVIII:C (IU dL^{-1})	×	×	×	×	×	×	×	×	×	×
aPTT (sec)	×	×	×	×	×	×	×	×	×	×
PCF (kdynes)	×	×	×	×	×	×	×	×	×	×
CEM (kdynes cm^{-2})	×	×	×	×	×	×	×	×	×	×
FOT (min)	×	×	×	×	×	×	×	×	×	×
R (min)	×	×	×	×	×	×	×	×	×	×
K (min)	×	×	×	×	×	×	×	×	×	×
MA (mm)	×	×	×	×	×	×	×	×	×	×
Plasma fibrinogen content (mg dL^{-1})	×									
Platelet count (L^{-1})	×									

3.4. ANALYTICAL TECHNIQUES:

Blood samples were processed for determination of the study variables using standardized analytical techniques as provided by the manufacturers (Table 3.3). Blood samples were processed in two laboratories. Plasma FVIII:C, aPTT, plasma fibrinogen content and platelet count were determined by the VCU Health System Department of Pathology Clinical Laboratory. Global hemostasis biomarkers (platelet function [PCF, CEM and FOT] and blood viscoelasticity [R, K and MA]) were determined in the Virginia Commonwealth University Coagulation Laboratory (VCAL). Plasma FVIII:C (based on one-stage clotting assay), aPTT and plasma fibrinogen content were measured using STart 4 (Diagnostica Stago Inc, Parsippany, NJ). Platelet count was measured using ADVIA 2120i (Siemens Healthcare Diagnostics Inc, Deerfield, IL). Platelet function biomarkers (PCF, CEM and FOT) were measured using HAS (Hemodyne Inc, Richmond, VA). Blood viscoelastic biomarkers (R, K and MA) were measured using TEG 5000 (Haemoscope Corp, Niles, IL).

Table 3.3. Analytical Techniques

Instrument	Variable
STart 4 (Diagnostica Stago Inc, Parsippany, NJ)	• Plasma FVIII:C (IU dL^{-1}) • aPTT (sec) • Plasma fibrinogen (mg dL^{-1})
ADVIA 2120i (Siemens Healthcare Diagnostics Inc, Deerfield, IL)	• Platelet count (L^{-1})
HAS (Hemodyne Inc, Richmond, VA)	• PCF (kdynes) • CEM (kdynes cm^{-2}) • FOT (min)
TEG 5000 (Haemoscope Corp, Niles, IL)	• R (min) • K (min) • MA (mm)

3.5. PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS:

3.5.1. Pharmacokinetic Modeling:

Plasma FVIII:C-time profiles over 48-h following rFVIII prophylactic dosing were obtained for each patient. NCA was performed on each individual profile to estimate pertinent PK parameters.[99] CA was performed after inspecting the terminal β phase (disposition part) of each individual profile using the following criteria: a monophasic decline trend as an indicator of the one-compartment body model; and a biphasic decline trend as an indicator of the two-compartment body model.[99] Accordingly, an appropriate CA model was fitted to plasma FVIII:C by non-linear regression. Pertinent PK parameters were estimated consequently. The choice of empiric weighing scheme, e.g., y^{-1}, to correct for residual errors was made upon visual inspection of residuals. The goodness of fit was evaluated by coefficient of determination (r^2) and model selection criterion (MSC). Estimated pertinent PK parameters along with associated standard deviation (SD) were evaluated as well.

3.5.2. Pharmacokinetic/Pharmacodynamic Modeling:

Following rFVIII prophylactic dosing, aPTT-time profiles over 48-h were obtained for each patient. In addition, global hemostasis biomarkers-time profiles over 48-h following rFVIII prophylactic dosing were obtained for each patient. Furthermore, aPTT-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing were obtained for each patient. Moreover, global hemostasis biomarkers-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing were obtained. Appropriate PK/PD modeling performed after inspecting each individual profile. Accordingly, plasma FVIII:C dependency on aPTT or global hemostasis biomarkers was fitted to an appropriate PK/PD model by non-linear regression. Pertinent PD parameters were estimated

consequently. The goodness of fit was evaluated by r^2 and MSC. Estimated pertinent PD parameters along with associated standard deviation (SD) were evaluated as well. All necessary computerized PK and PK/PD modeling tasks were performed using Scientist Version 2.0 (Micromath Research, Saint Louis, MO).

3.6. STATISTICAL ANALYSIS:

Appropriate descriptive statistics (mean [± SD], median [range] and count [%]) were used to characterize central tendency and dispersion. Correlation and analysis of variance (ANOVA), as appropriate, for pertinent PK and PD parameters were performed relative to clinically relevant modifiers of bleeding tendency (plasma fibrinogen content, platelet count, age, bodyweight, ethnicity and rFVIII prophylactic regimen) at baseline to assess possible source of PK and PD variability. Inter-group differences (frequent versus infrequent bleeders) in pertinent PK and PD parameters were assessed by ANOVA. *P* value <0.05 significance level was pre-specified for all statistical tests. All statistical analyses were performed using JMP Version 9.0.2 (SAS Institute Inc, Cary, NC).

CHAPTER 4. RESULTS

4.1. DEMOGRAPHICS:

Over 16 months, approximately 10 hemophiliacs were screened each month. Of these hemophiliacs, 9 eligible hemophiliacs enrolled in this study. The demographics of these patients are presented in Tables 4.1 and 4.2. As shown in Figure 4.1, 5 patients (55.6%) exhibited frequent bleeding tendency while 4 patients exhibited infrequent bleeding tendency (44.4%). The mean (±SD) age was 41.7 (±15.9) years, and the median (range) age was 40 (20–62) years. The mean (±SD) bodyweight was 79.1 (±14.3) kilograms (kg), and the median (range) bodyweight was 81 (61–107) kg. The majority of patients were Caucasians (6 [66.7%]) while the others were African Americans (2 [22.2%]) and Asian (1 [11.1%]). One patient (11.1%) had HBV. Seven patients (77.8%) had HCV. Five patients (55.6%) had HIV.

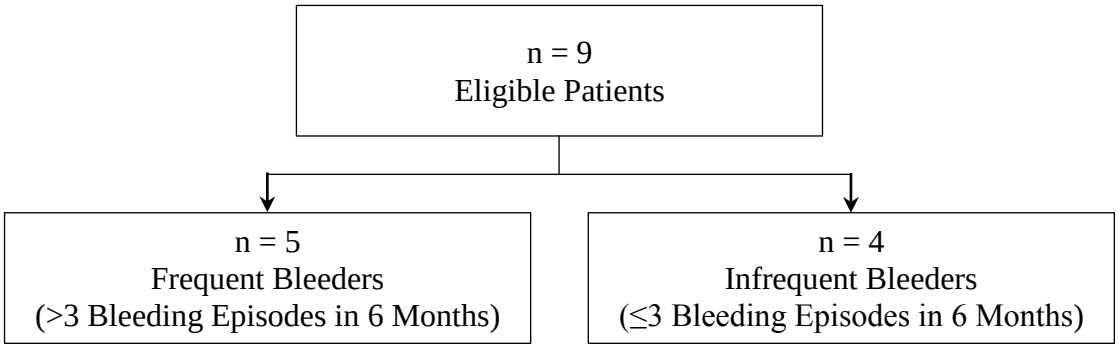
Table 4.1. Demographics

ID	Bleeding Tendency	Age (Year)	Bodyweight (kg)	Ethnicity	Comorbidity		
					HBV	HCV	HIV
Patient 1	Infrequent	62	107	African American	No	Yes	Yes
Patient 2	Infrequent	59	72	Caucasian	No	Yes	No
Patient 3	Infrequent	33	69	Asian	No	Yes	Yes
Patient 4	Frequent	46	83	Caucasian	No	Yes	Yes
Patient 5	Frequent	20	64	Caucasian	No	No	No
Patient 6	Frequent	60	61	Caucasian	No	Yes	Yes
Patient 7	Frequent	27	81	Caucasian	No	No	No
Patient 8	Frequent	28	86	Caucasian	No	Yes	No
Patient 9	Infrequent	40	88	African American	Yes	Yes	Yes

Table 4.2. Demographics Summary

Variable	Mean	(±SD)	Median	(Range)	Count	(%)
Bleeding tendency						
Frequent					5	(55.6)
Infrequent					4	(44.4)
Age (year)	41.7	(±15.9)	40	(20–62)		
Bodyweight (kg)	79.1	(±14.3)	81	(61–107)		
Ethnicity						
African American					2	(22.2)
Asian					1	(11.1)
Caucasian					6	(66.7)
Comorbidity						
HBV					1	(11.1)
HCV					7	(77.8)
HIV					5	(55.6)

Figure 4.1. Study Group Enrollment



4.2. RECOMBINANT FACTOR VIII PROPHYLAXIS:

Patients' rFVIII prophylaxis data are presented in Tables 4.3 and 4.4. The mean (±SD) rFVIII prophylactic dose was 2,544.8 (±955.0) IU, and the median (range) dose was 2,300.0 (1,560.0–4,464.0) IU. The mean (±SD) rFVIII prophylactic equivalent dose was 32.1 (±10.6) IU kg^{-1}, and the median (range) equivalent dose was 28.7 (21.4–54.4) IU kg^{-1}. Seven patients (77.8%) were on rFVIII prophylaxis two times per week while the other two patients (22.2%)

were on rFVIII prophylaxis three times per week. The majority of patients were on Kogenate FS (4 [44.4%]) while the others were on Advate (1 [11.1%], Helixate FS (2 [22.2%]) and Recombinate (2 [22.2%]).

Table 4.3. rFVIII Prophylaxis Data

ID	Dose (IU)	Equivalent Dose (IU kg^{-1})	Regimen (Times per Week)	Product
Patient 1	2,300.0	21.4	Two	Kogenate FS
Patient 2	2,058.0	28.7	Three	Kogenate FS
Patient 3	1,628.0	23.4	Three	Helixate FS
Patient 4	1,973.0	24.4	Two	Advate
Patient 5	2,400.0	36.3	Two	Helixate FS
Patient 6	1,560.0	25.6	Two	Recombinate
Patient 7	4,464.0	54.4	Two	Kogenate FS
Patient 8	3,520.0	40.9	Two	Recombinate
Patient 9	3,000.0	34.1	Two	Kogenate FS

Table 4.4. rFVIII Prophylaxis Data Summary

Variable	Mean	(±SD)	Median	(Range)	Count	(%)
Dose (IU)	2,544.8	(±955.0)	2,300.0	(1,560.0–4,464.0)		
Equivalent dose (IU kg^{-1})	32.1	(±10.6)	28.7	(21.4–54.4)		
Regimen (times per week)						
Two					7	(77.8)
Three					2	(22.2)
Product						
Advate					1	(11.1)
Helixate FS					2	(22.2)
Kogenate FS					4	(44.4)
Recombinate					2	(22.2)

4.3. PLASMA FIBRINOGEN CONTENT AND PLATELET COUNT:

Plasma fibrinogen content and platelet count for the patients are presented in Tables 4.5 and 4.6. The mean (±SD) plasma fibrinogen content was 334.9 (±52.4) mg dL^{-1}, and the median (range) plasma fibrinogen content was 328.0 (264.0–441.0) mg dL^{-1}. All patients had plasma fibrinogen content within the reference range (200.0–450.0 mg dL^{-1}).[100] The mean (±SD)

platelet count was 260.4 (±70.2) x 10^9 L^{-1}, and the median (range) plasma fibrinogen content was 261.0 (177.0–403.0) x 10^9 L^{-1}. All patients had platelet count within the reference range (140.0–440.0 x 10^9 L^{-1}).[101]

Table 4.5. Plasma Fibrinogen Content and Platelet Count

ID	Plasma Fibrinogen (mg dL^{-1})	Platelet Count (x10^9 L^{-1})
Patient 1	328.0	199.0
Patient 2	264.0	261.0
Patient 3	313.0	274.0
Patient 4	289.0	273.0
Patient 5	301.0	230.0
Patient 6	441.0	323.0
Patient 7	355.0	403.0
Patient 8	367.0	177.0
Patient 9	356.0	204.0

Table 4.6. Plasma Fibrinogen Content and Platelet Count Summary

Variable	Mean	(±SD)	Median	(Range)
Plasma fibrinogen (mg dL^{-1})	334.9	(±52.4)	328.0	(264.0–441.0)
Platelet count (x10^9 L^{-1})	260.4	(±70.2)	261.0	(177.0–403.0)

4.4. PHARMACOKINETIC MODELING:

4.4.1. Non-Compartmental Analysis:

Maximum concentration (C_{max}), V_{ss}, CL_{tot}, $t_{1/2}$, MRT and total area under the curve (AUC_∞) of the plasma FVIII:C-time profile from standard NCA are presented in Tables 4.7 and 4.8. The mean (±SD) C_{max} was 88.0 (±31.5) IU dL^{-1}, and the median (range) C_{max} was 90.0 (54.0–152.0) IU dL^{-1}. The time to maximum concentration (t_{max}) was 30 min for all patients. The mean (±SD) V_{ss} was 40.5 (±11.2) mL kg^{-1}, and the median (range) V_{ss} was 40.9 (22.8–58.2) mL kg^{-1}. The mean (±SD) CL_{tot} was 2.9 (±1.2) mL h^{-1} kg^{-1}, and the median (range) CL_{tot} was 3.0

(1.0–4.7) mL h^{-1} kg^{-1}. The mean (±SD) $t_{1/2}$ was 11.6 (±6.2) h, and the median (range) $t_{1/2}$ was 7.6 (6.8–23.8) h. The mean (±SD) MRT was 16.3 (±8.1) h, and the median (range) MRT was 12.3 (8.7–33.8) h. The mean (±SD) AUC_{∞} was 738.2 (±260.0) international unit per milliliter per minute (IU mL^{-1} min^{-1}), and the median (range) AUC_{∞} was 652.4 (472.7–1,325.5) IU mL^{-1} min^{-1}. The extrapolated area under the curve from last point to infinity (AUC_{extra}) of the plasma FVIII:C-time profile contributes to a small percentage of AUC_{∞}.

Table 4.7. NCA

ID	C_{max} (IU dL^{-1})	V_{ss} (mL kg^{-1})	CL_{tot} (mL h^{-1} kg^{-1})	$t_{1/2}$ (h)	MRT (h)	AUC_{∞} (IU mL^{-1} min^{-1})
Patient 1	70.0	32.8	1.0	23.8	33.8	1,325.5
Patient 2	68.0	35.5	3.0	7.3	11.9	576.9
Patient 3	55.0	58.2	2.3	19.3	25.0	606.4
Patient 4	116.0	22.8	1.5	12.0	15.4	964.8
Patient 5	92.0	41.8	3.8	7.6	11.0	590.7
Patient 6	54.0	55.8	3.2	13.4	17.3	472.7
Patient 7	152.0	40.9	4.7	6.9	8.7	703.8
Patient 8	90.0	43.0	3.8	7.4	11.4	652.4
Patient 9	95.0	33.4	2.7	6.8	12.3	750.8

Table 4.8. NCA Summary

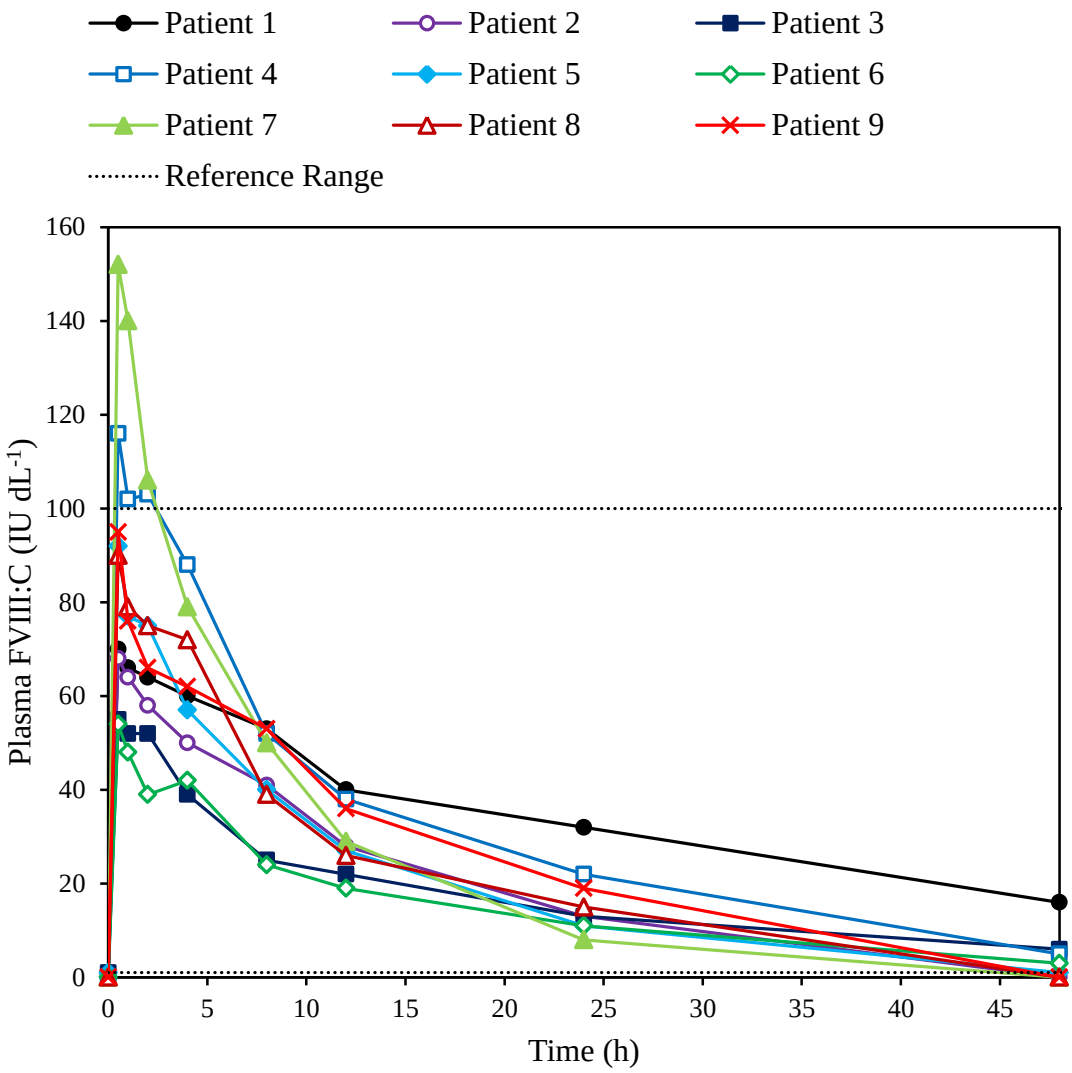
Parameter	Mean	(±SD)	Median	(Range)
C_{max} (IU dL^{-1})	88.0	(±31.5)	90.0	(54.0–152.0)
V_{ss} (mL kg^{-1})	40.5	(±11.2)	40.9	(22.8–58.2)
CL_{tot} (mL h^{-1} kg^{-1})	2.9	(±1.2)	3.0	(1.0–4.7)
$t_{1/2}$ (h)	11.6	(±6.2)	7.6	(6.8–23.8)
MRT (h)	16.3	(±8.1)	12.3	(8.7–33.8)
AUC_{∞} (IU mL^{-1} min^{-1})	738.2	(±260.0)	652.4	(472.7–1,325.5)

4.4.2. Compartmental Analysis:

Plasma FVIII:C-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.2) showed a monoexponetial decline trend in the terminal β phase (disposition part). On a logarithmic scale (Figure 4.3), these profiles showed a monophasic decline trend in the terminal

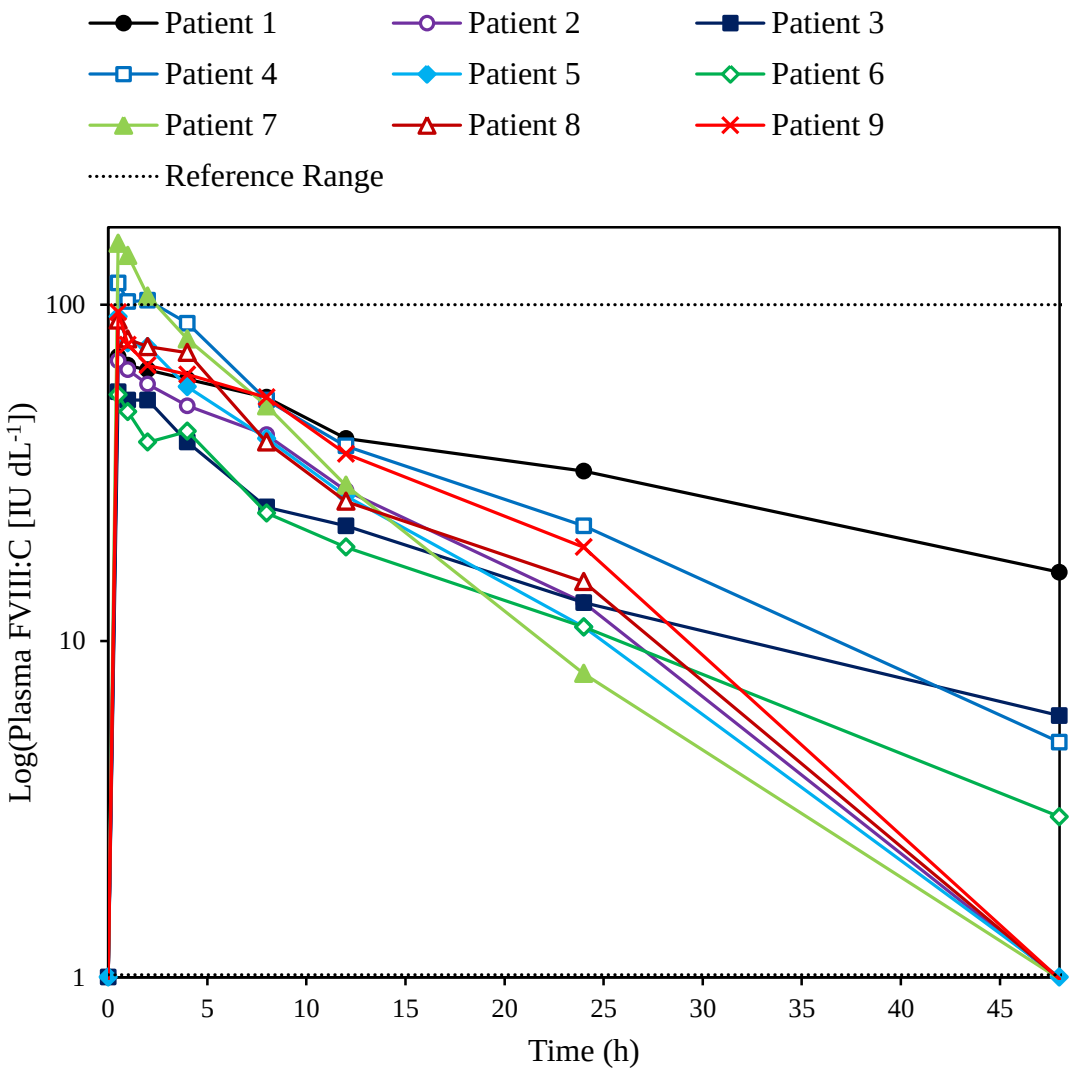
β phase (disposition part), and therefore the one-compartment body model was selected. The one-compartment body model assumes instant and homogenous distribution of FVIII, and therefore plasma FVIII:C represents FVIII:C in all other tissues.[99] Statistics and estimated PK parameters (V_{ss} and CL_{tot}) by fitting the one-compartment body model to plasma FVIII:C with y^{-1} empiric weighing scheme using non-linear regression are presented in Tables 4.9 and 4.10. The one-compartment body model showed a good fit as indicated by r^2 (0.976–0.999). MSC (2.86–5.41) indicated a negligible to medium degree of over-parameterization. There was a very high precision in V_{ss} and CL_{tot} estimation as indicated by very low associated SD. The mean (±SD) V_{ss} was 39.6 (±8.9) mL kg^{-1}, and the median (range) V_{ss} was 41.9 (21.0–51.3) mL kg^{-1}. The mean (±SD) CL_{tot} was 3.1 (±1.3) mL h^{-1} kg^{-1}, and the median (range) CL_{tot} was 3.0 (1.0–5.1) mL h^{-1} kg^{-1}.

Figure 4.2. Plasma FVIII:C-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Figure 4.3. Plasma FVIII:C-Time Profiles on a Logarithmic Scale[†]



[†]Individual profiles are presented in Appendices 5–13.

Table 4.9. One-Compartment Body Model

ID	r^2	MSC	V_{ss} (mL kg^{-1})	(±SD)	CL_{tot} (mL h^{-1} kg^{-1})	(±SD)
Patient 1	0.995	5.41	31.8	(±1.1)	1.0	(±0.1)
Patient 2	0.999	5.18	41.9	(±0.6)	3.0	(±0.1)
Patient 3	0.976	3.61	44.9	(±3.4)	2.8	(±0.3)
Patient 4	0.989	3.97	21.0	(±1.1)	1.6	(±0.1)
Patient 5	0.994	5.24	42.6	(±1.6)	4.0	(±0.2)
Patient 6	0.985	3.46	51.3	(±3.0)	3.6	(±0.3)
Patient 7	0.994	4.39	36.4	(±1.5)	5.1	(±0.2)
Patient 8	0.989	3.05	45.5	(±2.3)	4.1	(±0.3)
Patient 9	0.991	2.86	40.8	(±1.9)	2.7	(±0.2)

Table 4.10. One-Compartment Body Model Summary

Parameter	Mean	(±SD)	Median	(Range)
V_{ss} (mL kg^{-1})	39.6	(±8.9)	41.9	(21.0–51.3)
CL_{tot} (mL h^{-1} kg^{-1})	3.1	(±1.3)	3.0	(1.0–5.1)

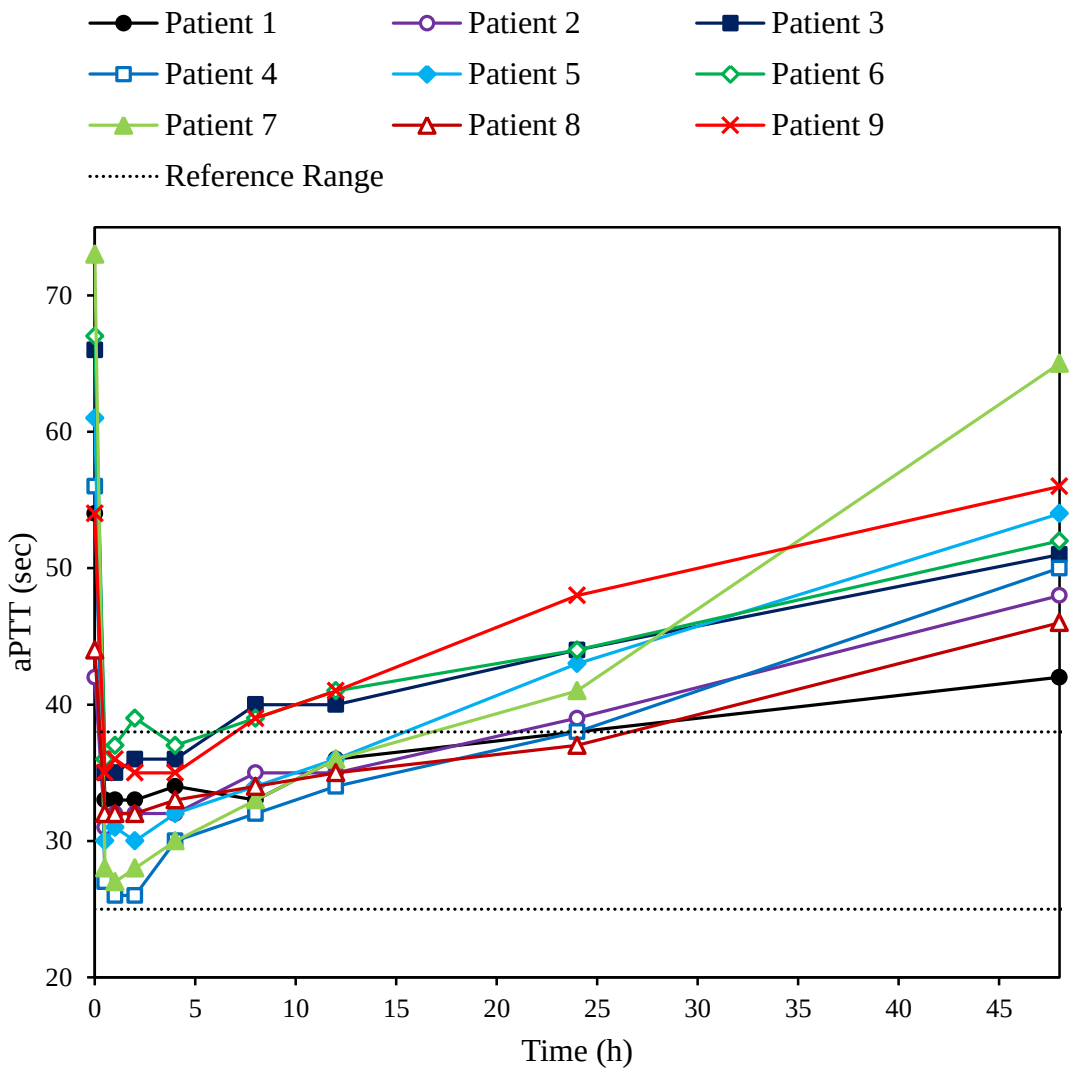
4.5. PHARMACOKINETIC/PHARMACODYNAMIC MODELING:

4.5.1. Activated Partial Thromboplastin Time:

Following rFVIII prophylactic dosing, aPTT-time profiles over 48-h (Figure 4.4) showed changing aPTT over time, which is a critical prerequisite for PK/PD modeling of aPTT. In addition, aPTT-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing (Figure 4.5) exhibited a negative linear trend, i.e., as plasma FVIII:C increased, the corresponding aPTT decreased. (The unit of aPTT is sec [very small unit]; subsequently, aPTT-plasma FVIII:C profiles [Figure 4.5] exhibited a negative linear trend rather than a clockwise hysteresis loop [which may be appeared at the first glance].) Therefore, aPTT was PK/PD modeled using the linear model that assumes reduction in the PD response of a drug with increasing its plasma level, i.e., increasing plasma FVIII:C is parallel to the corresponding aPTT reduction in the biophase (site of action [the intrinsic pathway in the cascade model of blood coagulation]).[75]

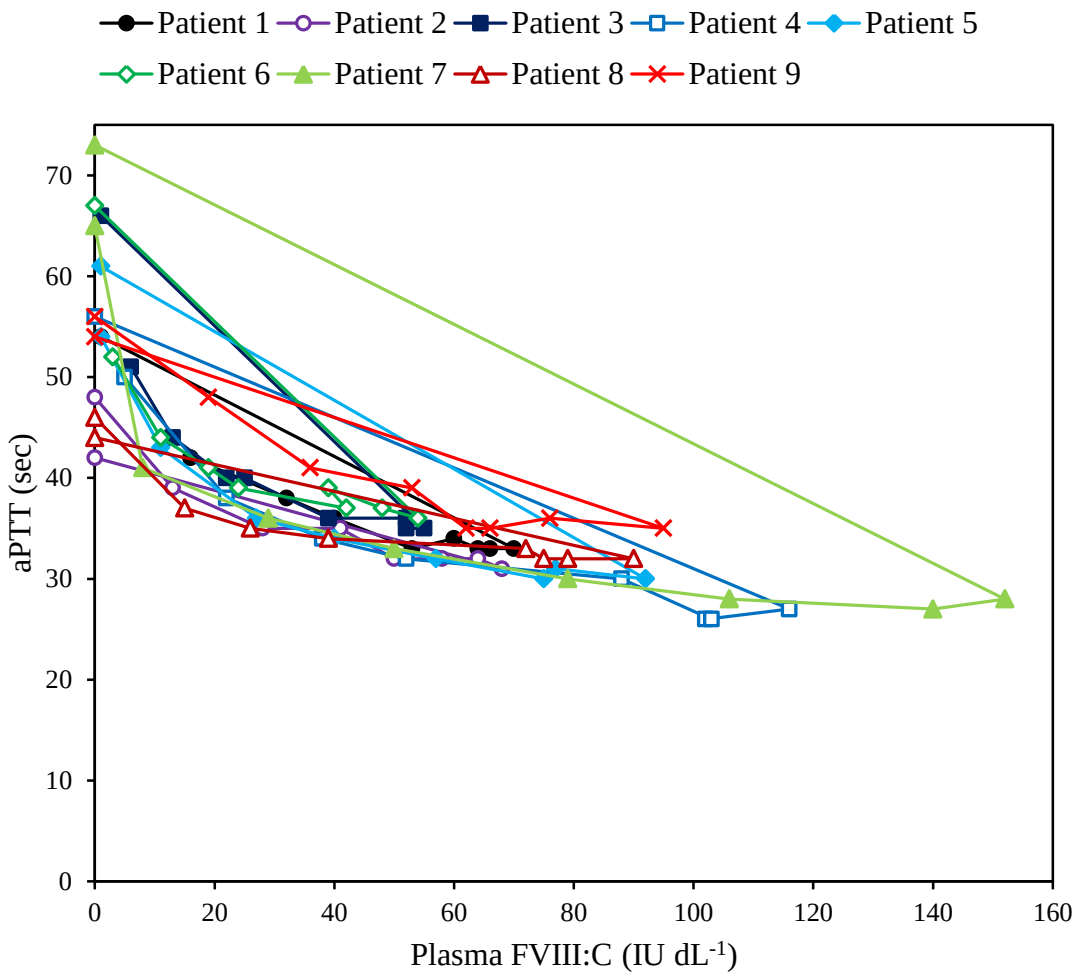
Only two PD parameters were estimated using the linear model, and therefore the linear model was a good choice for aPTT in order to reduce the degree of over-parameterization. Statistics and estimated PD parameters (baseline effect [E_0] and slope [a measure of aPTT sensitivity to changes in plasma FVIII:C]) by fitting plasma FVIII:C dependency on aPTT to the linear model using non-linear regression are presented in Tables 4.11 and 4.12. The linear model showed a good fit as indicated by r^2 (0.941–0.998). MSC (0.40–2.23) indicated a medium to high degree of over-parameterization. There was a very high precision in E_0 estimation as indicated by very low associated SD; however, there was a medium to high precision in slope estimation as indicated by low to medium associated SD. PK/PD linear modeling of aPTT showed reduction in the corresponding predicted aPTT with increasing the predicted plasma FVIII:C (Figures 4.6 and 4.7). The mean (±SD) E_0 was 48.9 (±4.4) sec, and the median (range) E_0 was 49.5 (41.5–53.9) sec. The mean (±SD) slope was -0.025 (±0.009) second deciliter per international unit (sec dL IU^{-1}), and the median (range) slope was -0.025 (-0.041– -0.013) sec dL IU^{-1}.

Figure 4.4. aPTT-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Figure 4.5. aPTT-Plasma FVIII:C Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

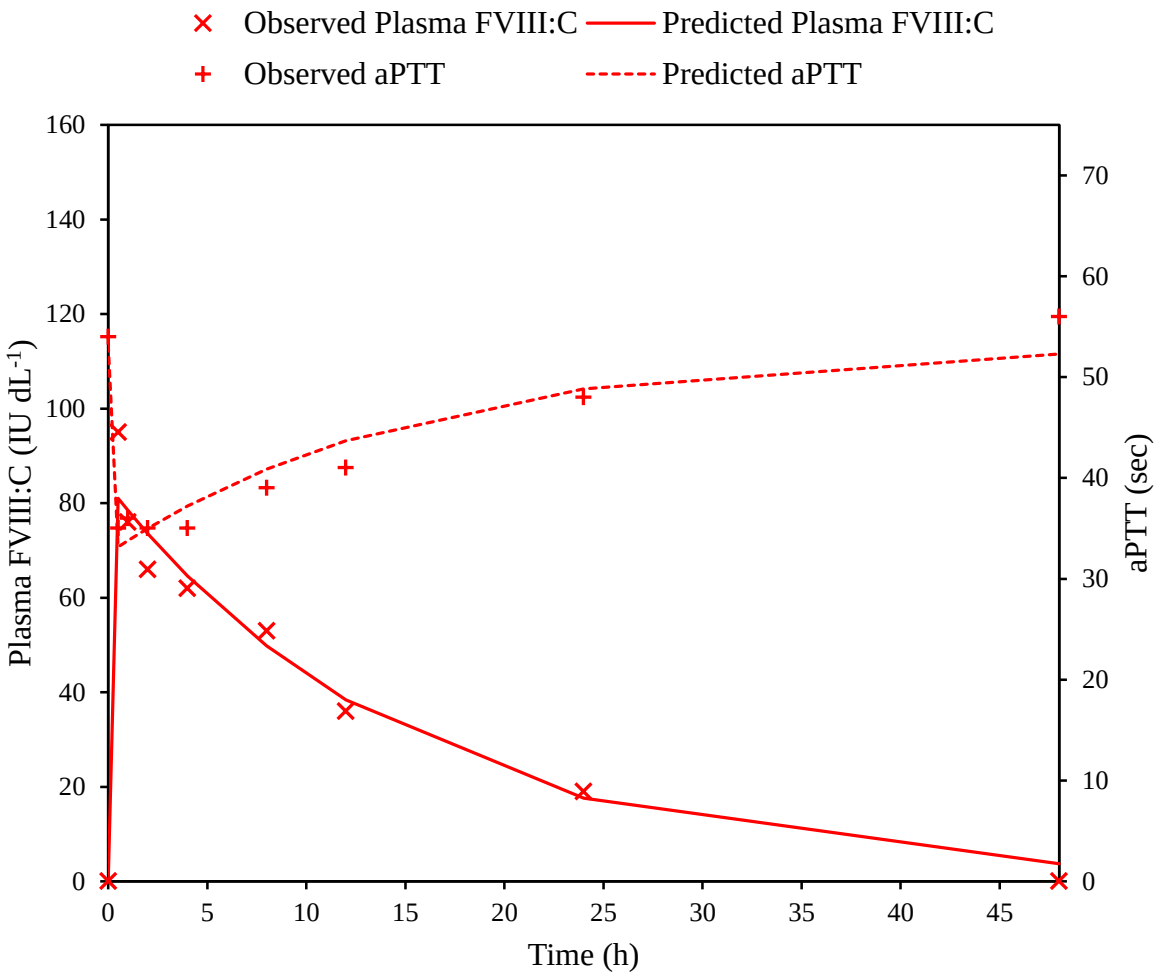
Table 4.11. aPTT PK/PD Linear Model

ID	r^2	MSC	E_0 (sec)	(±SD)	Slope (sec dL IU^{-1})	(±SD)
Patient 1	0.996	1.53	48.4	(±2.0)	-0.025	(±0.004)
Patient 2	0.996	1.39	43.1	(±1.4)	-0.019	(±0.003)
Patient 3	0.988	0.99	53.9	(±3.2)	-0.041	(±0.009)
Patient 4	0.988	1.39	47.2	(±2.6)	-0.021	(±0.004)
Patient 5	0.983	0.99	49.5	(±3.3)	-0.026	(±0.006)
Patient 6	0.986	0.70	53.4	(±3.6)	-0.039	(±0.011)
Patient 7	0.941	0.40	49.7	(±6.0)	-0.018	(±0.007)
Patient 8	0.996	1.03	41.5	(±1.5)	-0.013	(±0.003)
Patient 9	0.998	2.23	53.2	(±1.4)	-0.025	(±0.003)

Table 4.12. aPTT PK/PD Linear Model Summary

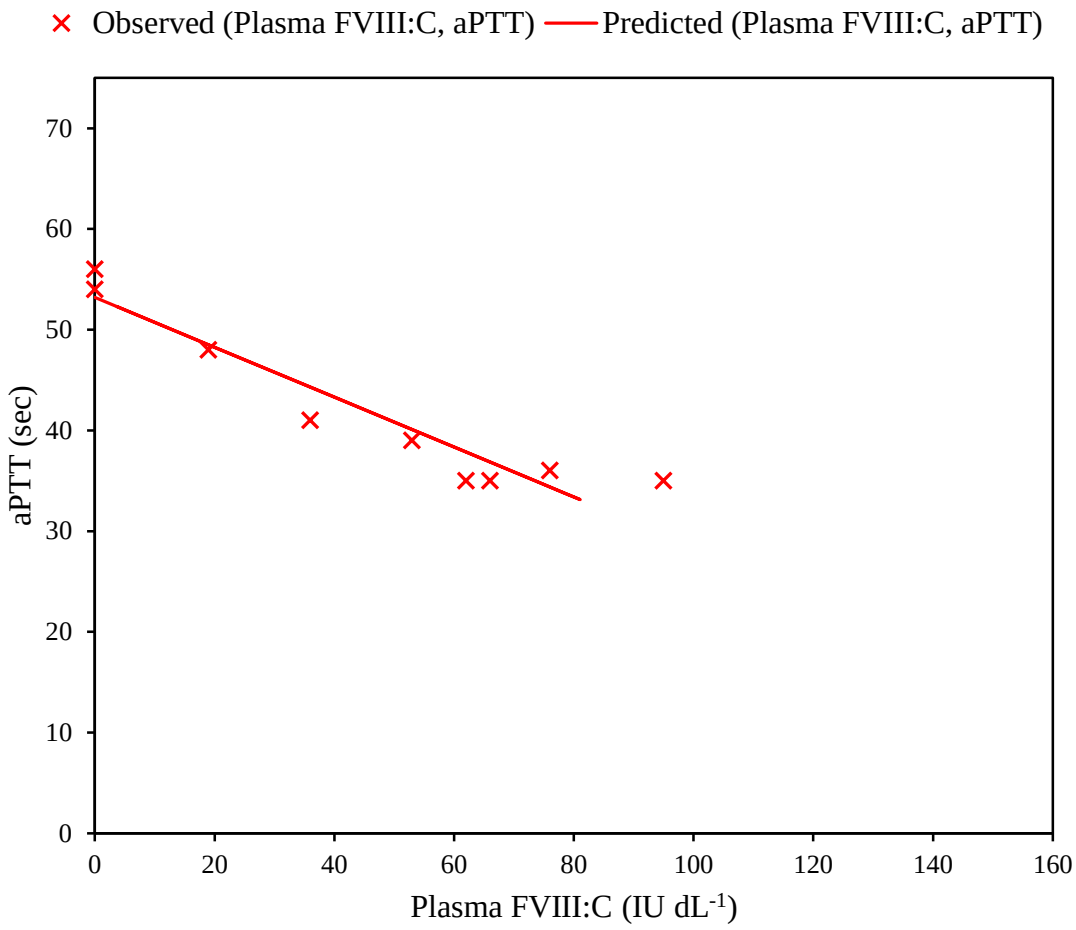
Parameter	Mean	(±SD)	Median	(Range)
E_0 (sec)	48.9	(±4.4)	49.5	(41.5–53.9)
Slope (sec dL IU^{-1})	-0.025	(±0.009)	-0.025	(-0.041– -0.013)

Figure 4.6. aPTT PK/PD Linear Model Time Dependent Profile for Patient 9[†]



[†]Other individual profiles are presented in Appendices 5–13.

Figure 4.7. aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile for Patient 9[†]



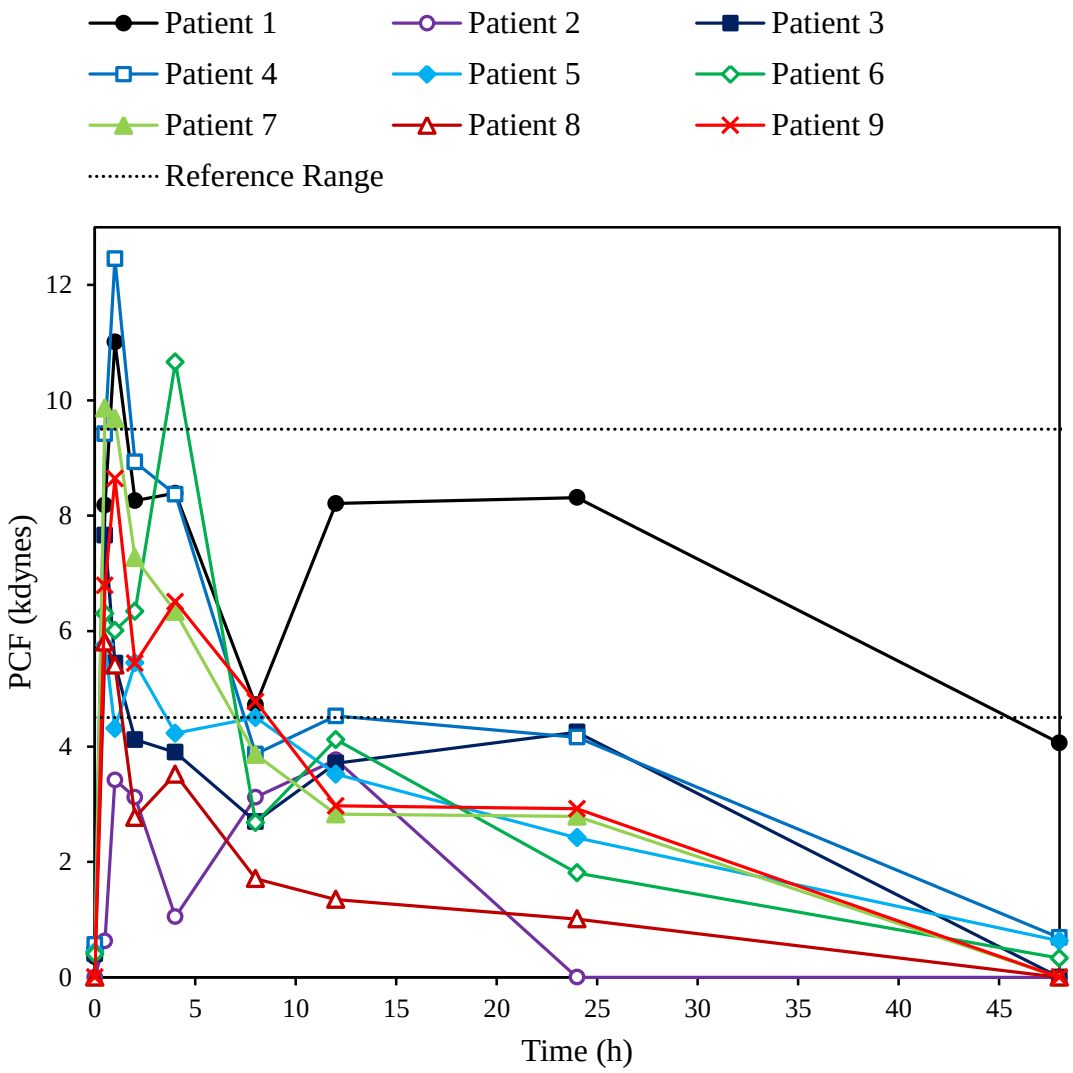
[†]Other individual profiles are presented in Appendices 5–13.

4.5.2. Platelet Contractile Force:

PCF-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.8) showed changing PCF over time, which is a critical prerequisite for PK/PD modeling of PCF. In addition, PCF-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing (Figure 4.9) exhibited a positive linear trend, i.e., as plasma FVIII:C increased, the corresponding PCF increased as well. Therefore, PCF was PK/PD modeled using the linear model that assumes increasing the PD response of a drug with increasing its plasma level, i.e., increasing plasma FVIII:C is parallel to the corresponding PCF increase in the biophase (site of action

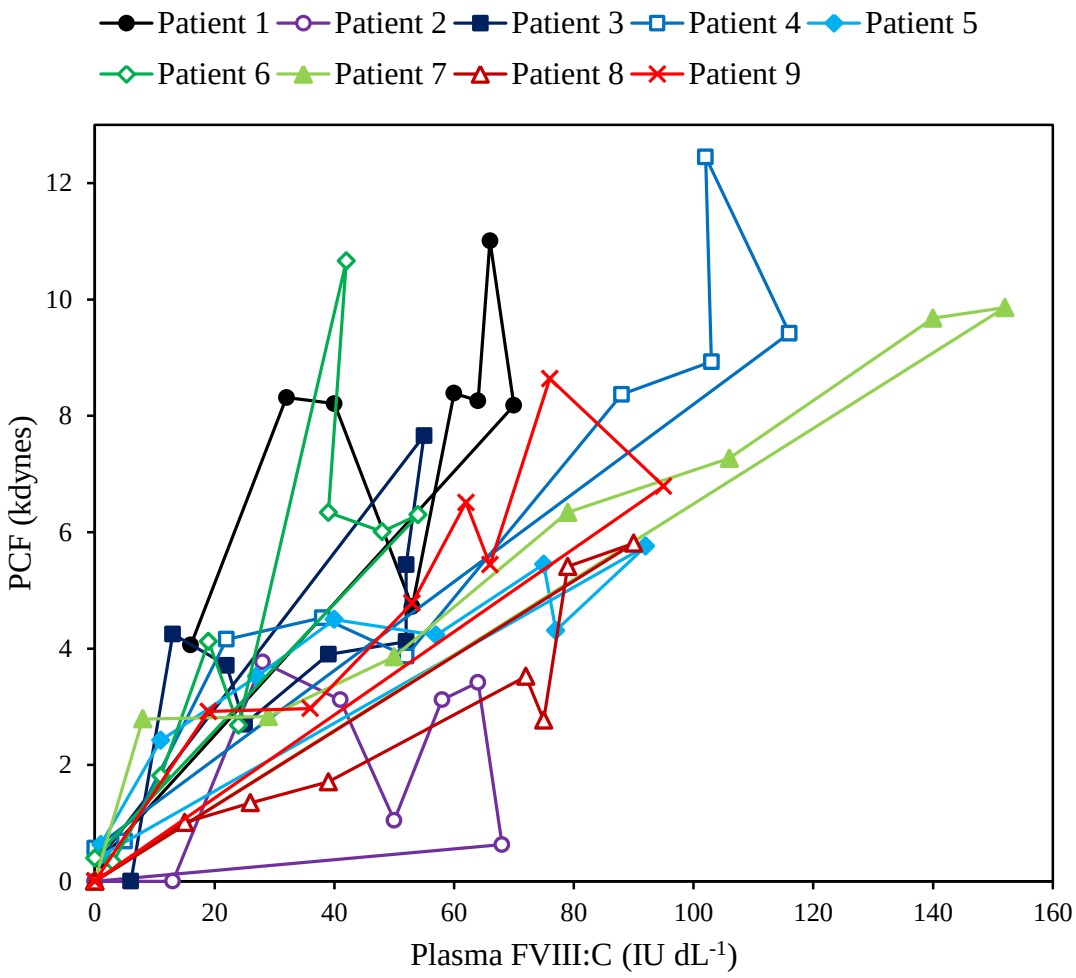
[platelet]).[75] Statistics and estimated PD parameters (E_0 and slope [a measure of PCF sensitivity to changes in plasma FVIII:C]) by fitting plasma FVIII:C dependency on PCF to the linear model using non-linear regression are presented in Tables 4.13 and 4.14. The linear model showed a good fit as indicated by r^2 (0.879–0.976), except Patient 2 (r^2 = 0.548). MSC (-0.43–2.26) indicated a medium to very high degree of over-parameterization. There was a very low precision in E_0 estimation as indicated by very high associated SD. Furthermore, there was a medium to high precision in slope estimation as indicated by medium to low associated SD, except Patient 2 (a very low precision in slope estimation as indicated by very high associated SD). PK/PD linear modeling of PCF showed increasing the corresponding predicted PCF with increasing the predicted plasma FVIII:C (Figures 4.10 and 4.11). The mean (±SD) E_0 was 0.3 (±0.3) kdynes, and the median (range) E_0 was 0.3 (0.0–0.7) kdynes. The mean (±SD) slope was 0.008 (±0.004) kilodynes deciliter per international unit (kdynes dL IU^{-1}), and the median (range) slope was 0.009 (0.002–0.014) kdynes dL IU^{-1}.

Figure 4.8. PCF-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Figure 4.9. PCF-Plasma FVIII:C Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

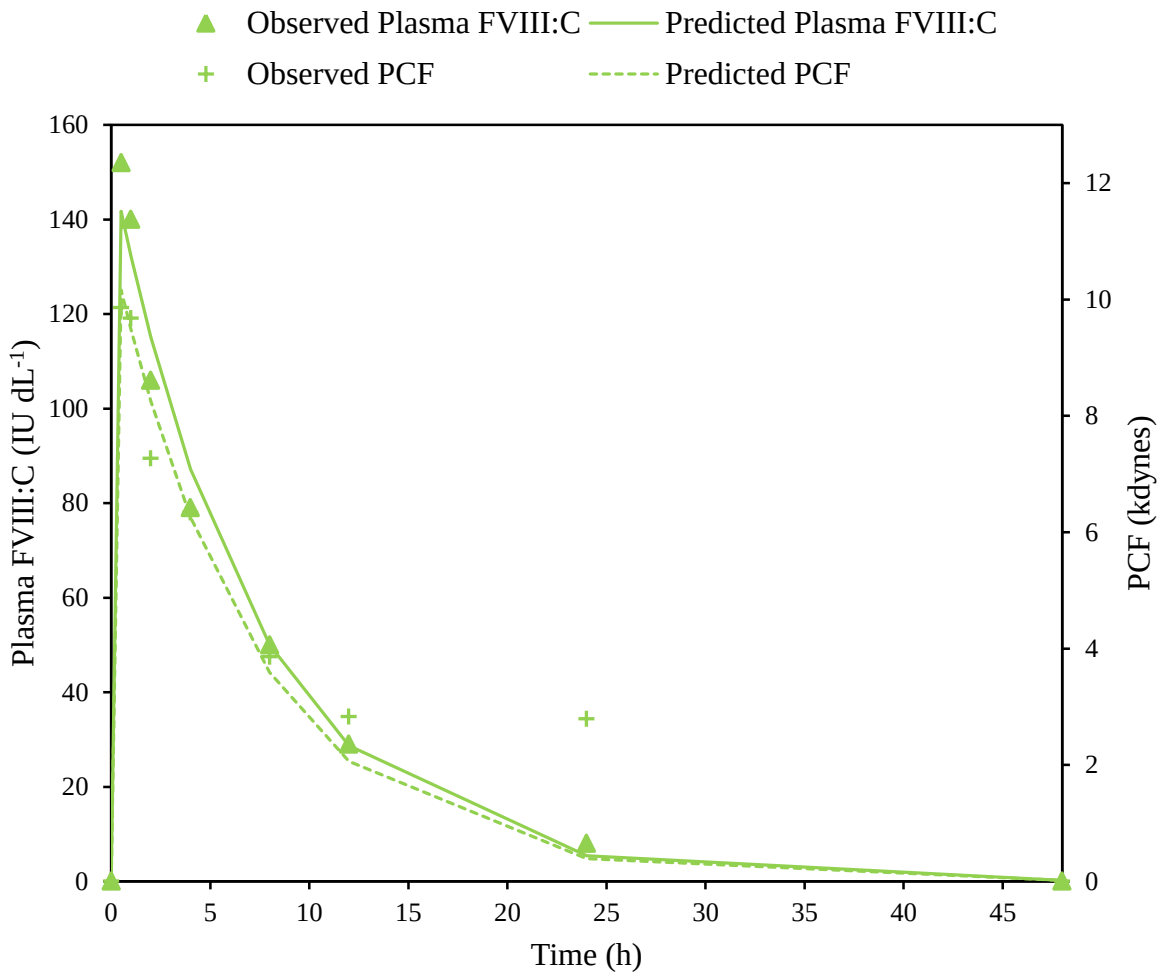
Table 4.13. PCF PK/PD Linear Model

ID	r^2	MSC	E_0 (±SD) (kdynes)		Slope (±SD) (kdynes dL IU^{-1})	
Patient 1	0.938	0.52	0.5	(±1.5)	0.013	(±0.003)
Patient 2	0.548	-0.43	0.0	(±1.0)	0.002	(±0.002)
Patient 3	0.908	0.67	0.6	(±0.9)	0.010	(±0.003)
Patient 4	0.963	1.65	0.6	(±0.9)	0.009	(±0.001)
Patient 5	0.956	1.15	0.7	(±0.5)	0.006	(±0.001)
Patient 6	0.879	0.63	0.3	(±1.2)	0.014	(±0.004)
Patient 7	0.976	2.26	0.0	(±0.5)	0.007	(±0.001)
Patient 8	0.942	1.53	0.0	(±0.5)	0.005	(±0.001)
Patient 9	0.971	1.92	0.0	(±0.6)	0.009	(±0.001)

Table 4.14. PCF PK/PD Linear Model Summary

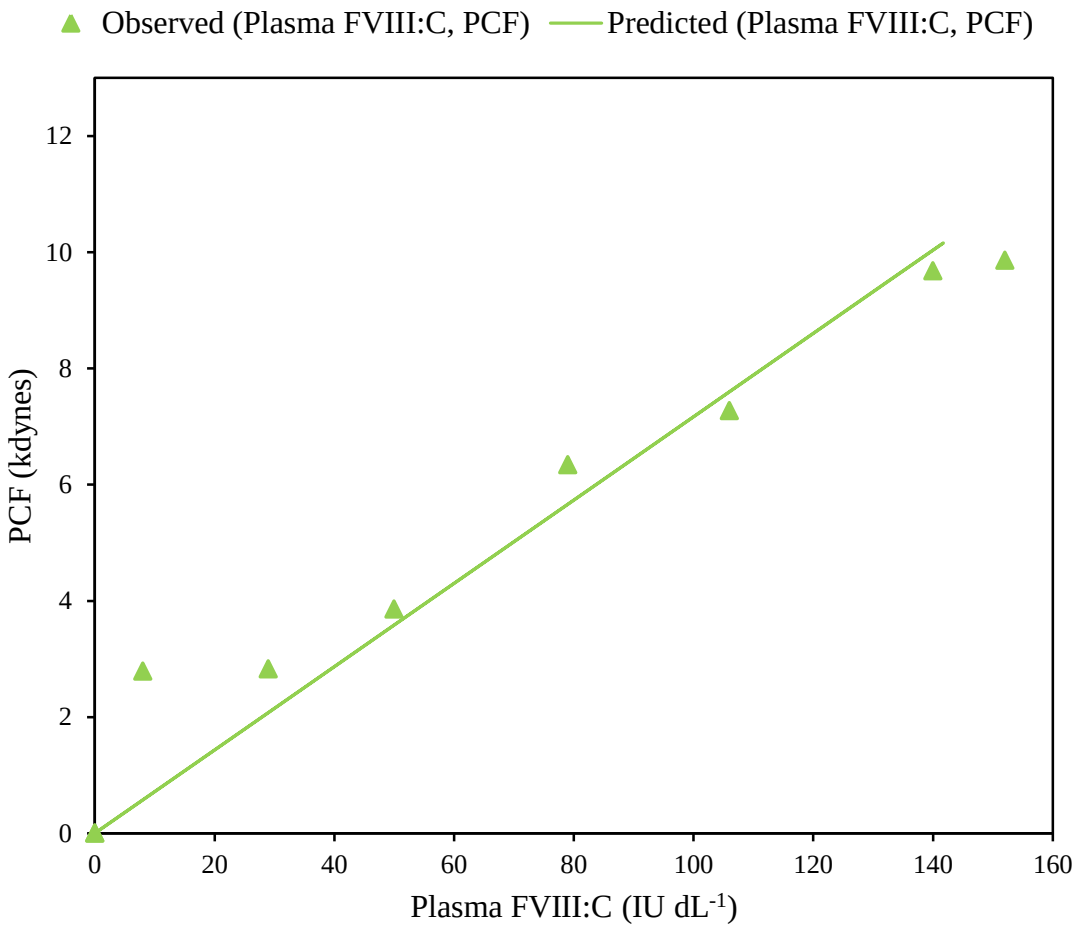
Parameter	Mean	(±SD)	Median	(Range)
E_0 (kdynes)	0.3	(±0.3)	0.3	(0.0–0.7)
Slope (kdynes dL IU^{-1})	0.008	(±0.004)	0.009	(0.002–0.014)

Figure 4.10. PCF PK/PD Linear Model Time Dependent Profile for Patient 7[†]



[†]Other individual profiles are presented in Appendices 5–13.

Figure 4.11. PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile for Patient 7[†]



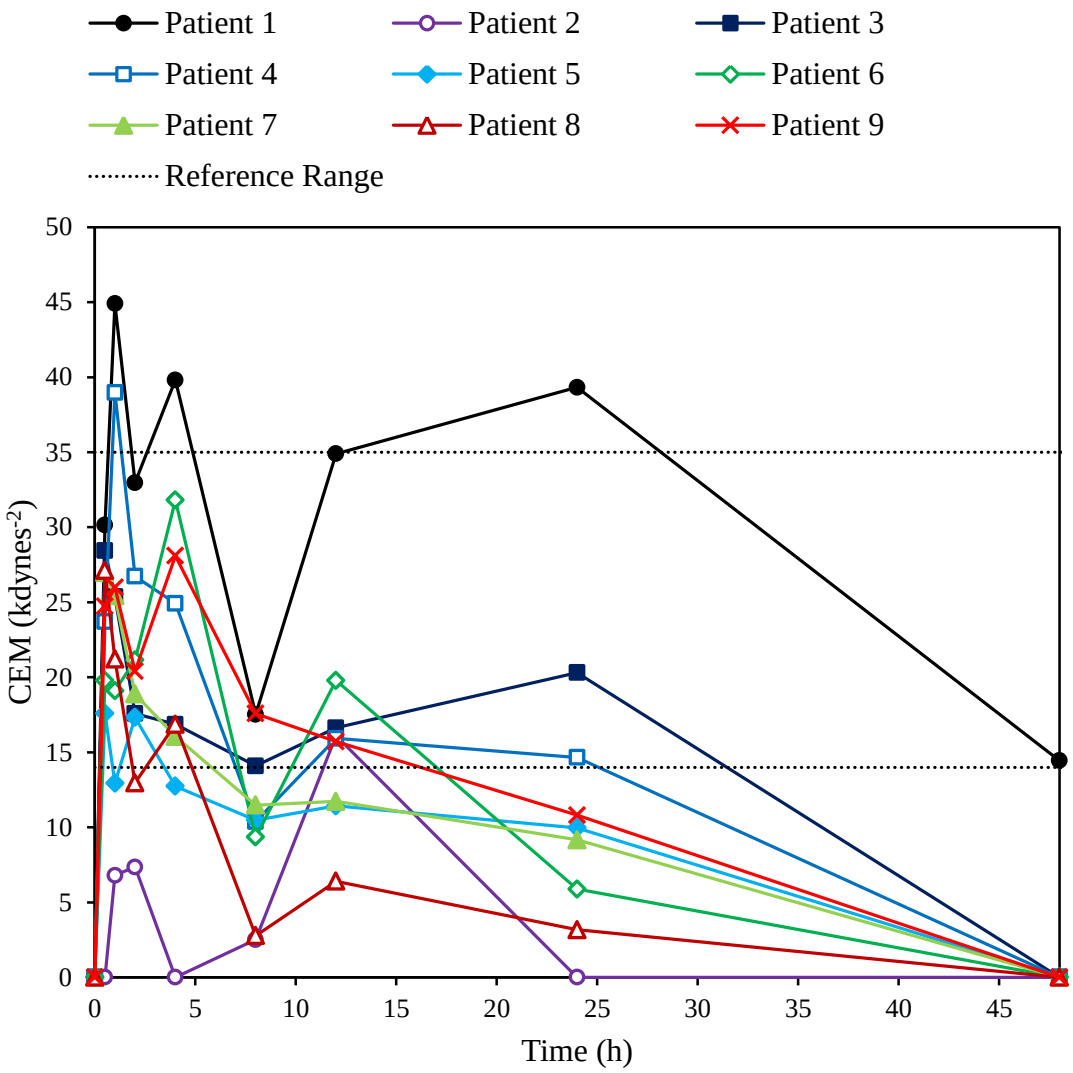
[†]Other individual profiles are presented in Appendices 5–13.

4.5.3. Clot Elastic Modulus:

CEM-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.12) showed changing CEM over time, which is a critical prerequisite for PK/PD modeling of CEM. In addition, CEM-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing (Figure 4.13) exhibited a positive linear trend, i.e., as plasma FVIII:C increased, the corresponding CEM increased as well. Therefore, CEM was PK/PD modeled using the linear model that assumes increasing the PD response of a drug with increasing its plasma level, i.e., increasing plasma FVIII:C is parallel to the corresponding CEM increase in the biophase (site of action

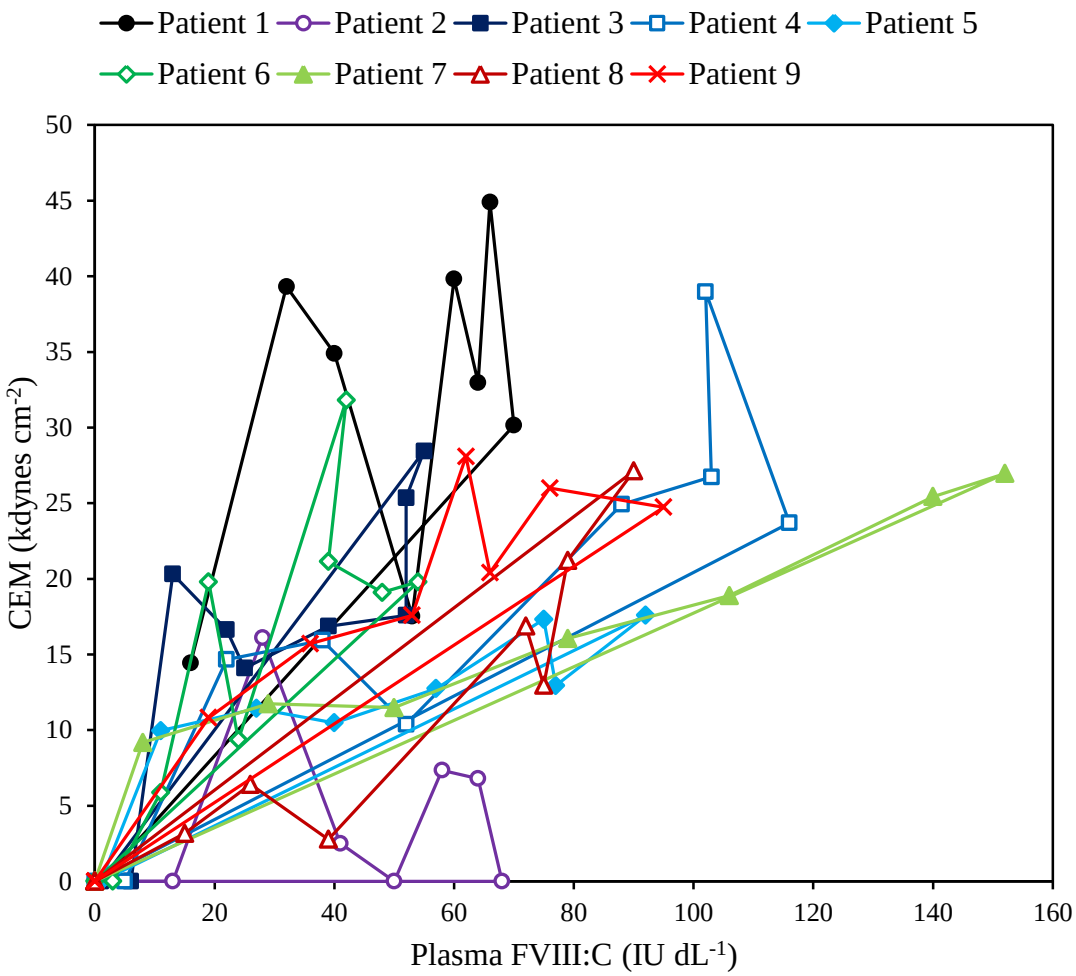
[platelet]).[75] Statistics and estimated PD parameters (E_0 and slope [a measure of CEM sensitivity to changes in plasma FVIII:C]) by fitting plasma FVIII:C dependency on CEM to the linear model using non-linear regression are presented in Tables 4.15 and 4.16. The linear model showed a good fit as indicated by r^2 (0.868–0.969), except Patient 2 (r^2 = 0.298). MSC (-0.49–1.74) indicated a medium to very high degree of over-parameterization. There was a very low precision in E_0 estimation as indicated by very high associated SD. Furthermore, there was a medium to high precision in slope estimation as indicated by low to medium associated SD, except Patient 2 (a very low precision in slope estimation as indicated by very high associated SD). PK/PD linear modeling of CEM showed increasing the corresponding predicted CEM with increasing the predicted plasma FVIII:C (Figures 4.14 and 4.15). The mean (±SD) E_0 was 0.0 (±0.0) kdynes cm^{-2}, and the median (range) E_0 was 0.0 (0.0–0.0) kdynes cm^{-2}. The mean (±SD) slope was 0.032 (±0.016) kilodynes deciliter per international unit per square centimeter (kdynes dL IU^{-1} cm^{-2}), and the median (range) slope was 0.027 (0.011–0.056) kdynes dL IU^{-1} cm^{-2}.

Figure 4.12. CEM-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Figure 4.13. CEM-Plasma FVIII:C Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

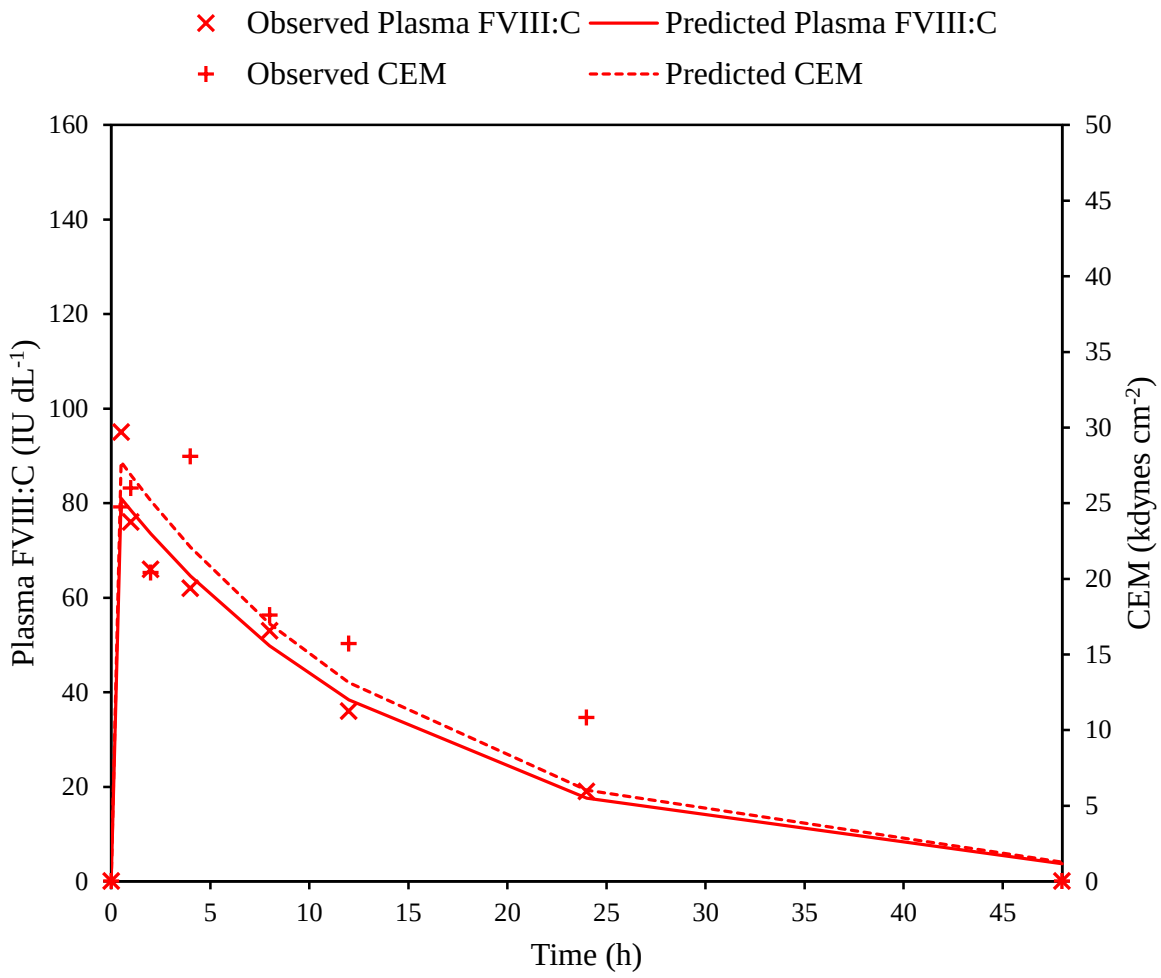
Table 4.15. CEM PK/PD Linear Model

ID	r^2	MSC	E_0 (kdynes cm^{-2})	(±SD)	Slope (kdynes dL IU^{-1} cm^{-2})	(±SD)
Patient 1	0.899	0.20	0.0	(±8.3)	0.056	(±0.017)
Patient 2	0.298	-0.49	0.0	(±3.6)	0.011	(±0.008)
Patient 3	0.902	0.55	0.0	(±3.9)	0.049	(±0.011)
Patient 4	0.924	1.02	0.0	(±3.8)	0.027	(±0.005)
Patient 5	0.908	0.59	0.0	(±2.2)	0.022	(±0.004)
Patient 6	0.883	0.64	0.0	(±4.0)	0.048	(±0.013)
Patient 7	0.949	1.39	0.0	(±2.1)	0.019	(±0.003)
Patient 8	0.868	0.81	0.0	(±3.1)	0.018	(±0.006)
Patient 9	0.969	1.74	0.0	(±2.3)	0.034	(±0.004)

Table 4.16. CEM PK/PD Linear Model Summary

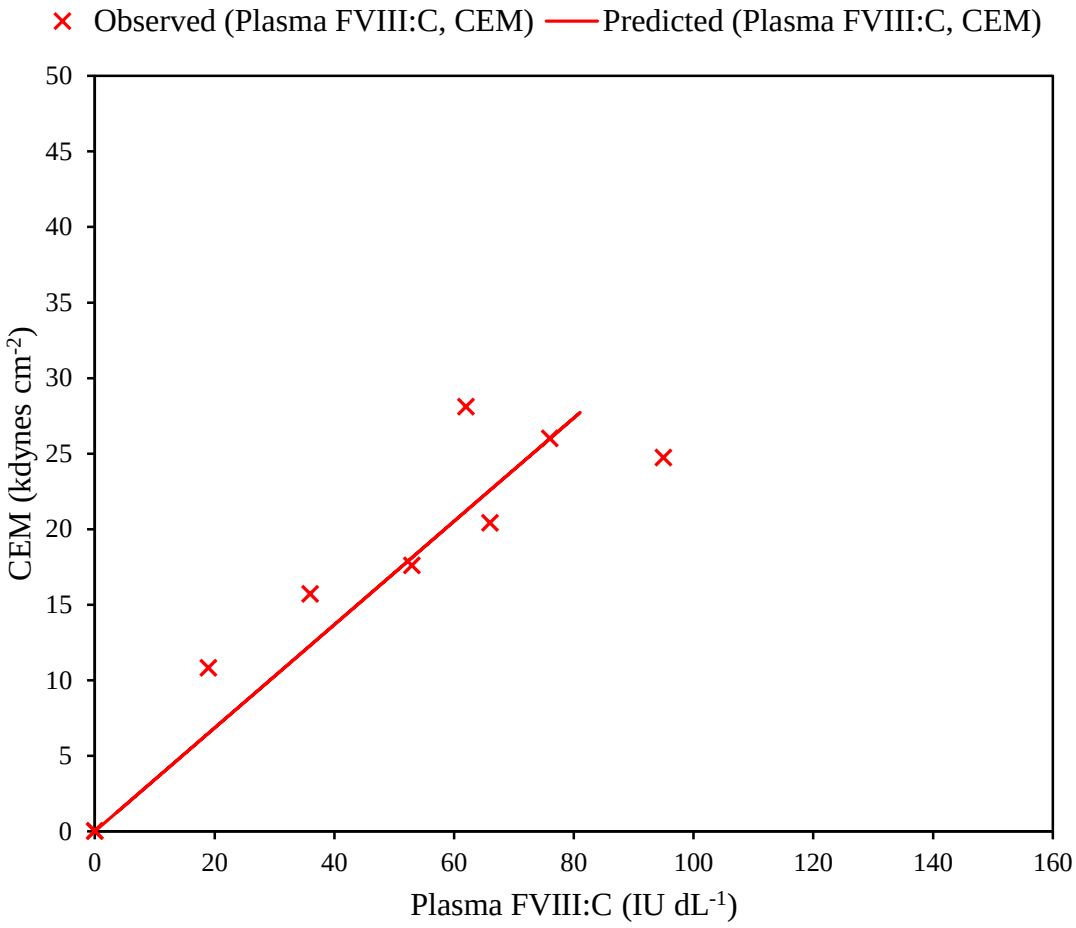
Parameter	Mean	(±SD)	Median	(Range)
E_0 (kdynes cm^{-2})	0.0	(±0.0)	0.0	(0.0–0.0)
Slope (kdynes dL IU^{-1} cm^{-2})	0.032	(±0.016)	0.027	(0.011–0.056)

Figure 4.14. CEM PK/PD Linear Model Time Dependent Profile for Patient 9[†]



[†]Other individual profiles are presented in Appendices 5–13.

Figure 4.15. CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile for Patient 9[†]



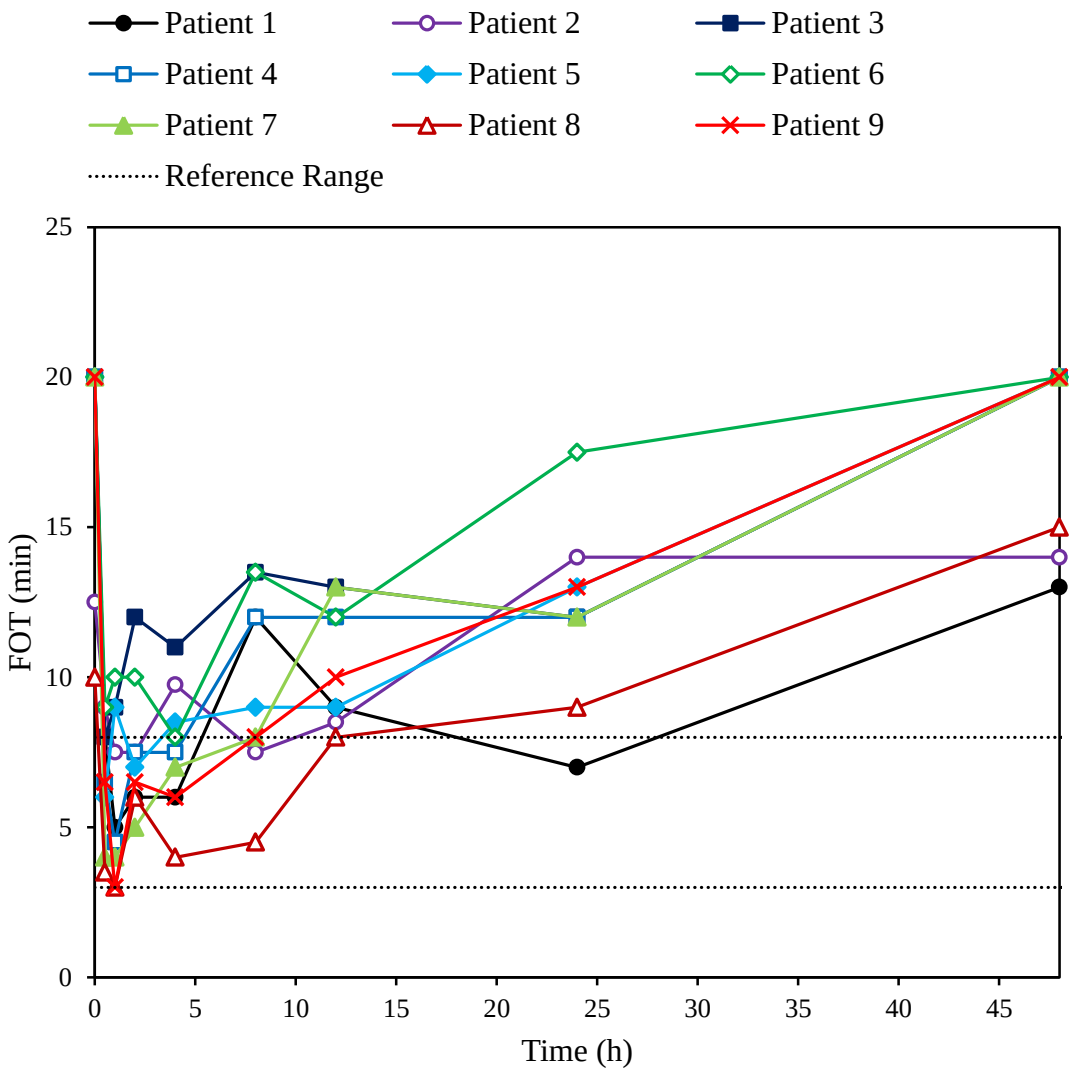
[†]Other individual profiles are presented in Appendices 5–13.

4.5.4. Force Onset Time:

FOT-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.16) showed changing FOT over time, which is a critical prerequisite for PK/PD modeling of FOT. In addition, FOT-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing (Figure 4.17) exhibited a clockwise hysteresis loop, i.e., as plasma FVIII:C increased, the reduction in FOT was delayed. Therefore, FOT was PK/PD modeled using the sigmoidal maximum effect (E_{max}) model that assumes a delay in the PD response of a drug with increasing its plasma level, i.e., increasing plasma FVIII:C corresponds to a delayed reduction in FOT in the biophase (site

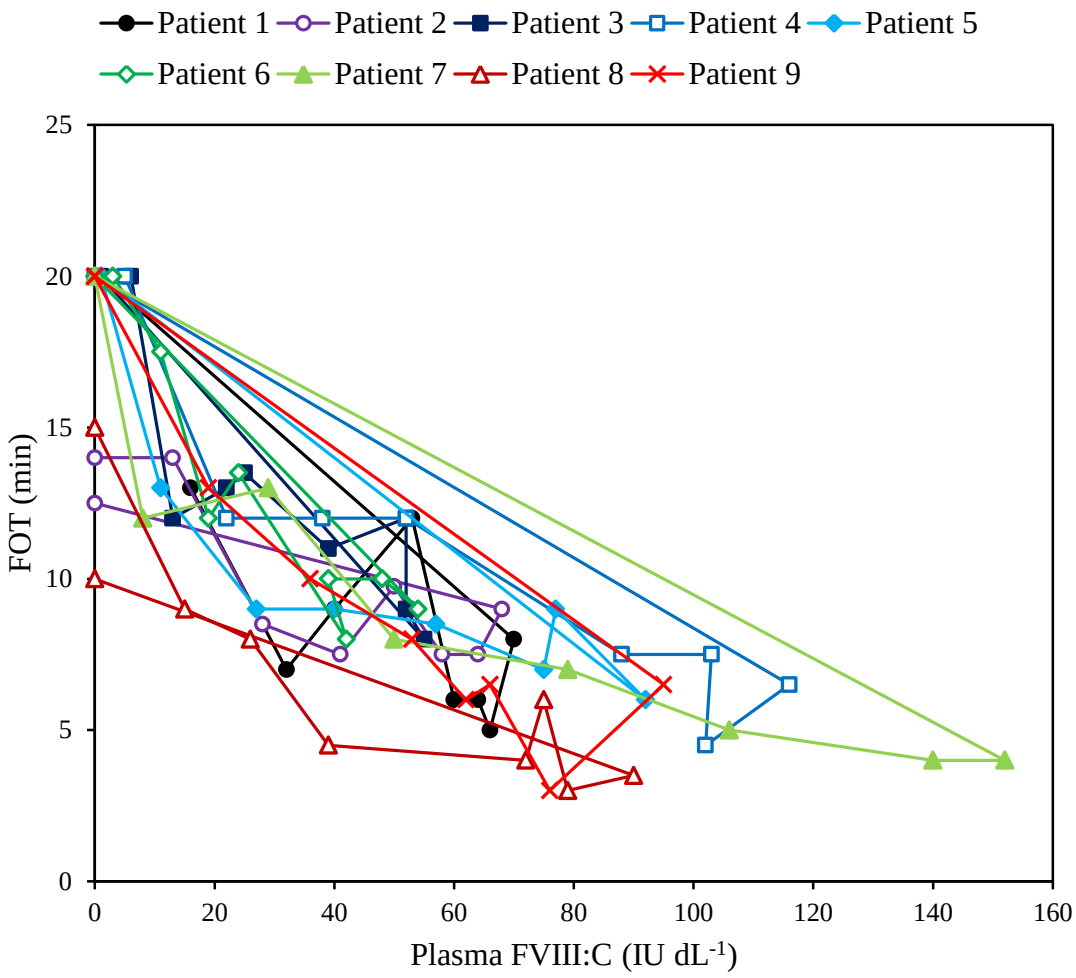
of action [platelet]).[75] Statistics and estimated PD parameters (E_0, E_{max}, half the maximum effective concentration [EC_{50}] and Hill coefficient [n (a measure of FOT-plasma FVIII:C curve steepness)]) by fitting plasma FVIII:C dependency on FOT to the sigmoidal E_{max} model using non-linear regression are presented in Tables 4.17 and 4.18. The sigmoidal E_{max} model showed a good fit as indicated by r^2 (0.947–0.995). MSC (-0.02–2.68) indicated a medium to very high degree of over-parameterization. There was a high precision in E_0 estimation as indicated by low associated SD. There was a low precision in E_{max} estimation as indicated by high associated SD, except Patient 5 (a high precision in E_{max} as indicated by low associated SD). There was a very low precision in EC_{50} estimation as indicated by very high associated SD. The upper limit for the EC_{50} was set as the C_{max}, which may have affected proper estimation of EC_{50}. There was a very low precision in n estimation as indicated by very high associated SD. PK/PD sigmoidal E_{max} modeling of FOT showed a delayed reduction in the corresponding predicted FOT with increasing the predicted plasma FVIII:C (Figures 4.18 and 4.19). The mean (±SD) E_0 was 19.8 (±3.7) min, and the median (range) E_0 was 21.0 (12.7–23.2) min. The mean (±SD) E_{max} was -14.1 (±4.8) min, i.e., the mean (±SD) E_{max} was reduced by 70.1 (±16.9) %. The median (range) E_{max} was -14.3 (-20.7– -5.9) min, i.e., the median (range) E_{max} was reduced by 70.6 (41.9–98.4) %. The mean (±SD) EC_{50} was 87.8 (±31.4) IU dL^{-1}, and the median (range) EC_{50} was 90.0 (54.0–152.0) IU dL^{-1}. The mean (±SD) n was 1.11 (±0.29), and the median (range) n was 1.10 (0.59–1.46).

Figure 4.16. FOT-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Figure 4.17. FOT-Plasma FVIII:C Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Table 4.17. FOT PK/PD Sigmoidal E_{max} Model

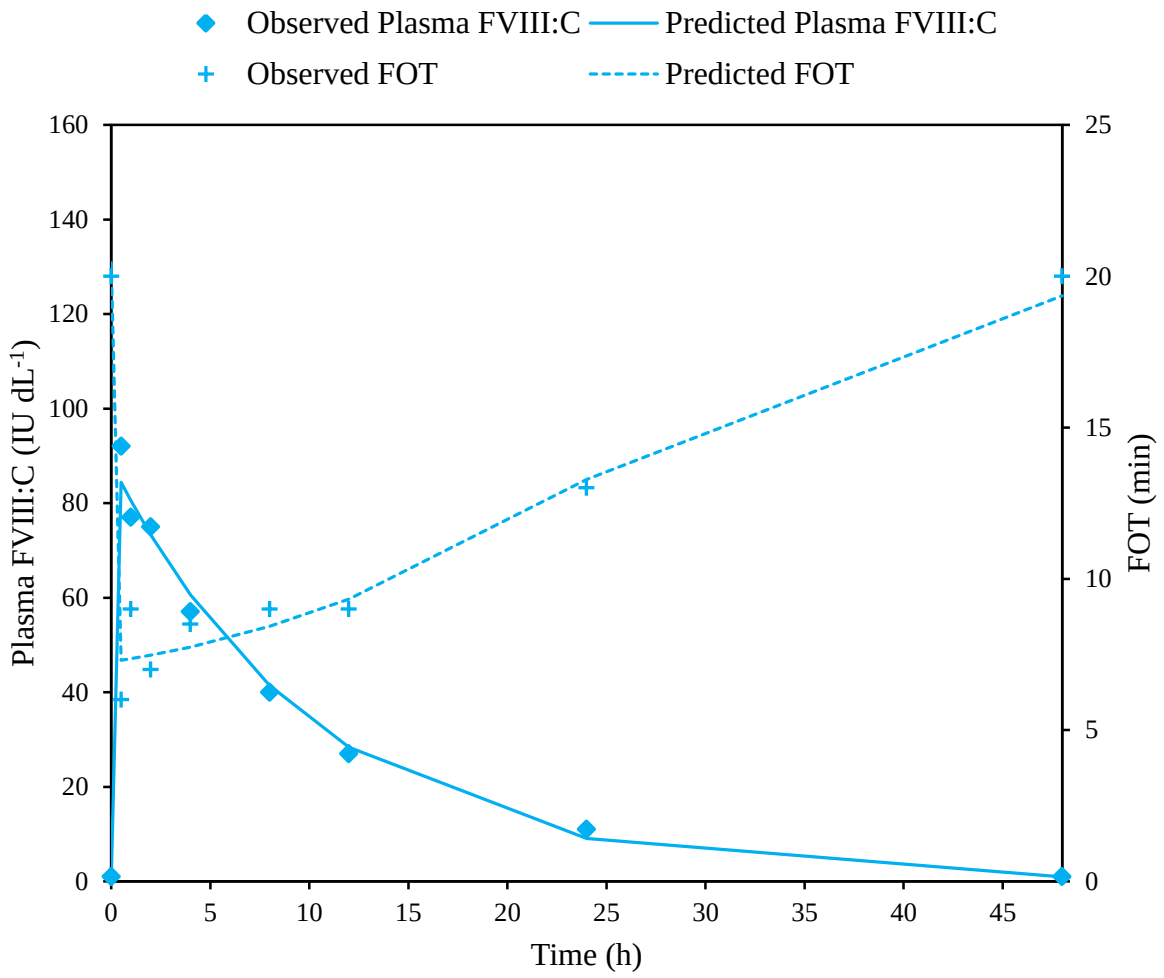
ID	r^2	MSC	E_0 (min)	(±SD)	E_{max} (min)	(±SD)
Patient 1	0.959	0.60	20.9	(±2.9)	-16.0	(±21.6)
Patient 2	0.974	-0.02	14.1	(±2.2)	-5.9	(±3.4)
Patient 3	0.985	0.84	21.2	(±2.3)	-11.2	(±3.3)
Patient 4	0.972	0.98	22.2	(±2.8)	-17.2	(±7.1)
Patient 5	0.995	2.68	20.4	(±1.1)	-14.3	(±2.6)
Patient 6	0.980	0.73	22.1	(±2.6)	-13.1	(±5.3)
Patient 7	0.980	1.65	21.0	(±2.1)	-20.7	(±11.6)
Patient 8	0.947	0.52	12.7	(±2.2)	-9.0	(±4.5)
Patient 9	0.970	1.17	23.2	(±2.8)	-19.3	(±6.0)

ID	E_{max} (% Reduction)	EC_{50} (IU dL^{-1})	(±SD)	n	(±SD)
Patient 1	76.6	70.0	(±103.5)	0.88	(±4.59)
Patient 2	41.9	68.0	(±99.7)	1.43	(±2.28)
Patient 3	52.8	55.0	(±41.6)	1.46	(±1.25)
Patient 4	77.7	116.0	(±136.2)	0.92	(±0.79)
Patient 5	69.8	90.0	(±34.5)	1.10	(±0.51)
Patient 6	59.4	54.0	(±56.5)	1.13	(±1.09)
Patient 7	98.4	152.0	(±304.9)	0.59	(±0.48)
Patient 8	70.6	90.0	(±88.7)	1.36	(±2.23)
Patient 9	83.4	95.0	(±76.3)	1.09	(±0.81)

Table 4.18. FOT PK/PD Sigmoidal E_{max} Model Summary

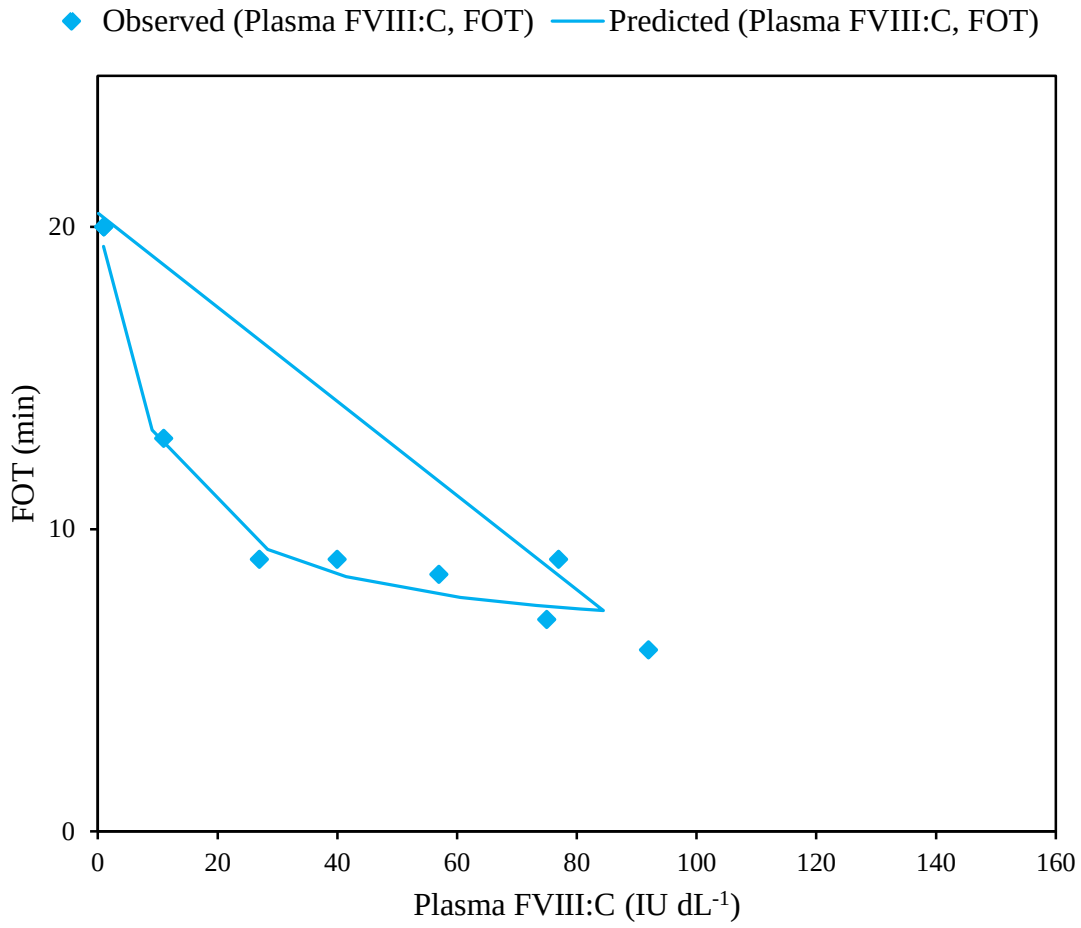
Parameter	Mean	(±SD)	Median	(Range)
E_0 (min)	19.8	(±3.7)	21.0	(12.7–23.2)
E_{max} (min)	-14.1	(±4.8)	-14.3	(-20.7– -5.9)
E_{max} (% Reduction)	70.1	(±16.9)	70.6	(41.9–98.4)
EC_{50} (IU dL^{-1})	87.8	(±31.4)	90.0	(54.0–152.0)
n	1.11	(±0.29)	1.10	(0.59–1.46)

Figure 4.18. FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile for Patient 5[†]



[†]Other individual profiles are presented in Appendices 5–13.

Figure 4.19. FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile for Patient 5[†]



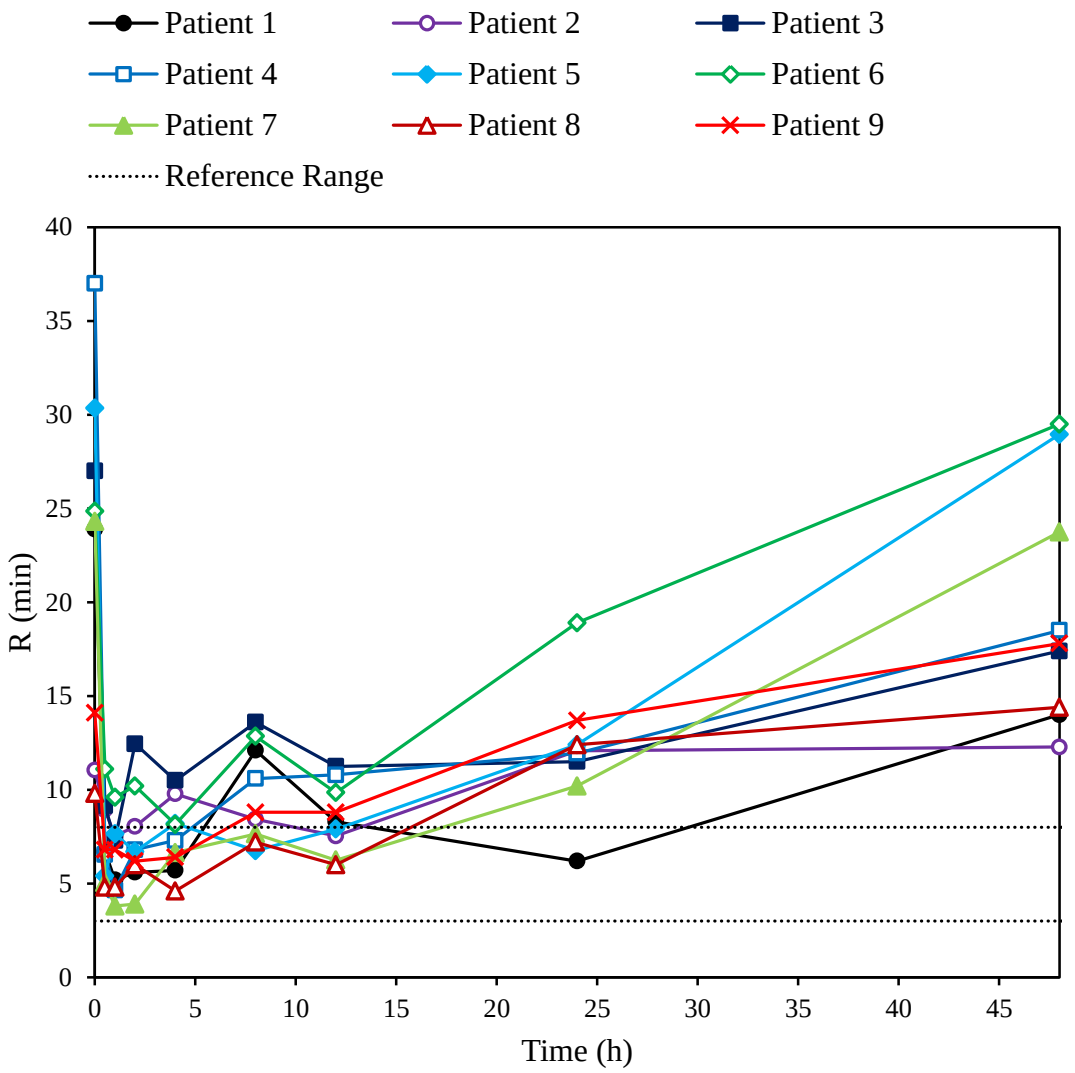
[†]Other individual profiles are presented in Appendices 5–13.

4.5.5. Reaction-Time:

R-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.20) showed changing R over time, which is a critical prerequisite for PK/PD modeling of R. In addition, R-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing (Figure 4.21) exhibited a clockwise hysteresis loop, i.e., as plasma FVIII:C increased, the reduction in R was delayed. Therefore, R was PK/PD modeled using the sigmoidal E_{max} model that assumes a delay in the PD response of a drug with increasing its plasma level, i.e., increasing plasma FVIII:C corresponds to a delayed reduction in R in the biophase (site of action [clot]).[75] Statistics and

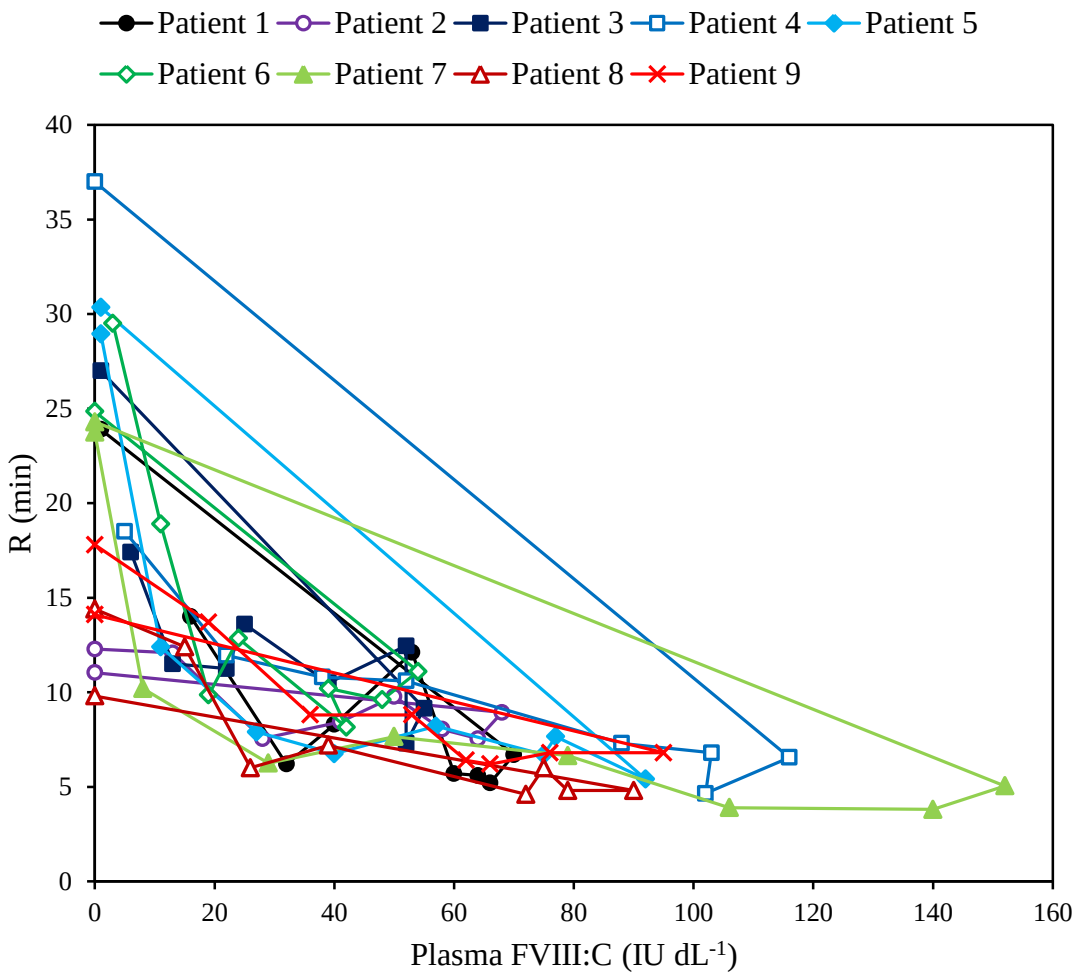
estimated PD parameters (E_0, E_{max}, EC_{50} and n [a measure of R-plasma FVIII:C curve steepness]) by fitting plasma FVIII:C dependency on R to the sigmoidal E_{max} model using non-linear regression are presented in Tables 4.19 and 4.20. The sigmoidal E_{max} model showed a good fit as indicated by r^2 (0.952–0.998). MSC (-0.13–4.21) indicated a negligible to very high degree of over-parameterization. There was a high precision in E_0 estimation as indicated by low associated SD. There was a low precision in E_{max} estimation as indicated by high associated SD, except Patients 5 and 7 (a high precision in E_{max} as indicated by low associated SD). There was a very low precision in EC_{50} estimation as indicated by very high associated SD, except Patient 5 (a medium precision in EC_{50} estimation as indicated by medium associated SD). The upper limit for the EC_{50} was set as the C_{max}, which may have affected proper estimation of EC_{50}. There was a very low precision in n estimation as indicated by very high associated SD, except Patient 5 (a medium precision in n estimation as indicated by medium associated SD). PK/PD sigmoidal E_{max} modeling of R showed a delayed reduction in the corresponding predicted R with increasing the predicted plasma FVIII:C (Figures 4.22 and 4.23). The mean (±SD) E_0 was 23.9 (±8.4) min, and the median (range) E_0 was 25.1 (12.1–37.1) min. The mean (±SD) E_{max} was -19.6 (±12.5) min, i.e., the mean (±SD) E_{max} was reduced by 74.9 (±26.0) %. The median (range) E_{max} was -20.4 (-46.3– -3.7) min, i.e., the median (range) E_{max} was reduced by 78.9 (30.6–124.6) %. The mean (±SD) EC_{50} was 68.5 (±28.4) IU dL^{-1}, and the median (range) EC_{50} was 68.0 (22.8–116.0) IU dL^{-1}. The mean (±SD) n was 1.20 (±0.58), and the median (range) n was 1.49 (0.31–1.85).

Figure 4.20. R-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Figure 4.21. R-Plasma FVIII:C Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Table 4.19. R PK/PD Sigmoidal E_{max} Model

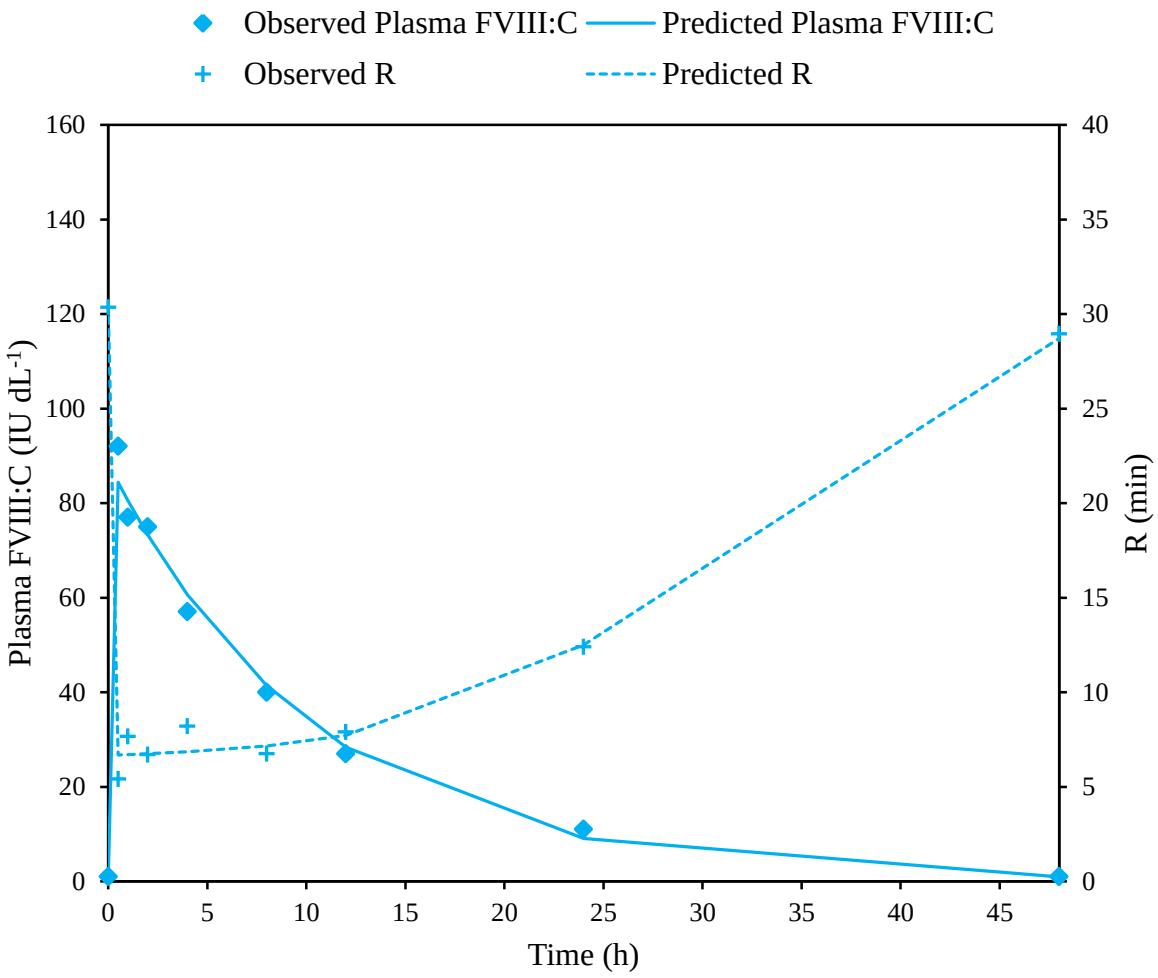
ID	r^2	MSC	E_0 (min)	(±SD)	E_{max} (min)	(±SD)
Patient 1	0.960	0.99	24.8	(±3.0)	-20.4	(±16.5)
Patient 2	0.984	-0.13	12.1	(±1.6)	-3.7	(±2.2)
Patient 3	0.987	1.52	27.1	(±2.2)	-24.3	(±33.8)
Patient 4	0.995	3.29	37.1	(±1.6)	-46.3	(±33.3)
Patient 5	0.998	4.21	30.5	(±1.0)	-24.1	(±1.2)
Patient 6	0.979	1.29	28.5	(±3.2)	-18.7	(±4.3)
Patient 7	0.992	2.91	25.1	(±1.5)	-21.2	(±2.4)
Patient 8	0.952	0.31	12.5	(±2.1)	-7.3	(±3.7)
Patient 9	0.964	0.47	17.0	(±2.7)	-10.2	(±4.1)

ID	E_{max} (% Reduction)	EC_{50} (IU dL^{-1})	(±SD)	n	(±SD)
Patient 1	82.1	70.0	(±83.8)	1.01	(±3.81)
Patient 2	30.6	68.0	(±105.8)	1.62	(±2.94)
Patient 3	89.9	55.0	(±378.3)	0.42	(±0.86)
Patient 4	124.6	116.0	(±564.3)	0.31	(±0.27)
Patient 5	78.9	45.5	(±8.4)	1.58	(±0.34)
Patient 6	65.6	54.0	(±31.5)	1.85	(±1.43)
Patient 7	84.5	22.8	(±11.4)	0.78	(±0.26)
Patient 8	57.9	90.0	(±87.5)	1.73	(±4.22)
Patient 9	59.8	95.0	(±93.0)	1.49	(±1.65)

Table 4.20. R PK/PD Sigmoidal E_{max} Model Summary

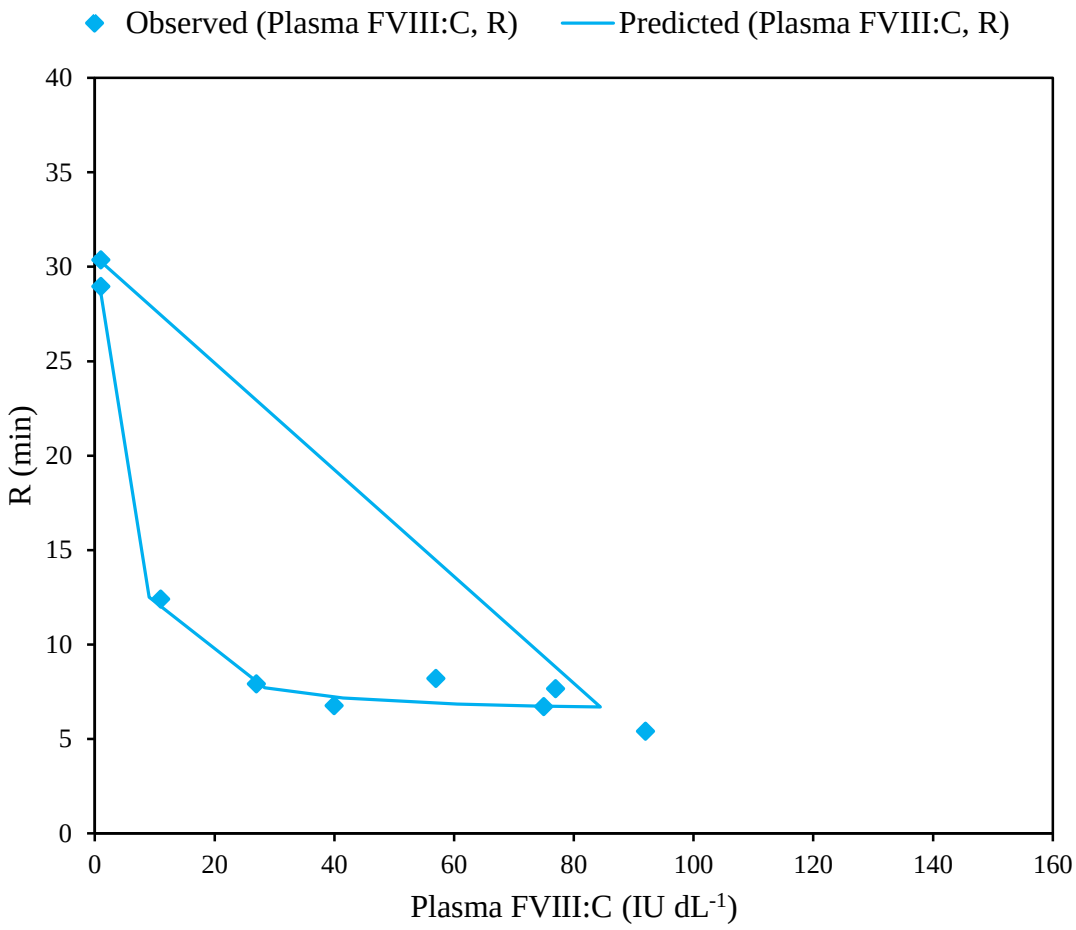
Parameter	Mean	(±SD)	Median	(Range)
E_0 (min)	23.9	(±8.4)	25.1	(12.1–37.1)
E_{max} (min)	-19.6	(±12.5)	-20.4	(-46.3– -3.7)
E_{max} (% Reduction)	74.9	(±26.0)	78.9	(30.6–124.6)
EC_{50} (IU dL^{-1})	68.5	(±28.4)	68.0	(22.8–116.0)
n	1.20	(±0.58)	1.49	(0.31–1.85)

Figure 4.22. R PK/PD Sigmoidal E_{max} Model Time Dependent Profile for Patient 5[†]



[†]Other individual profiles are presented in Appendices 5–13.

Figure 4.23. R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile for Patient 5[†]



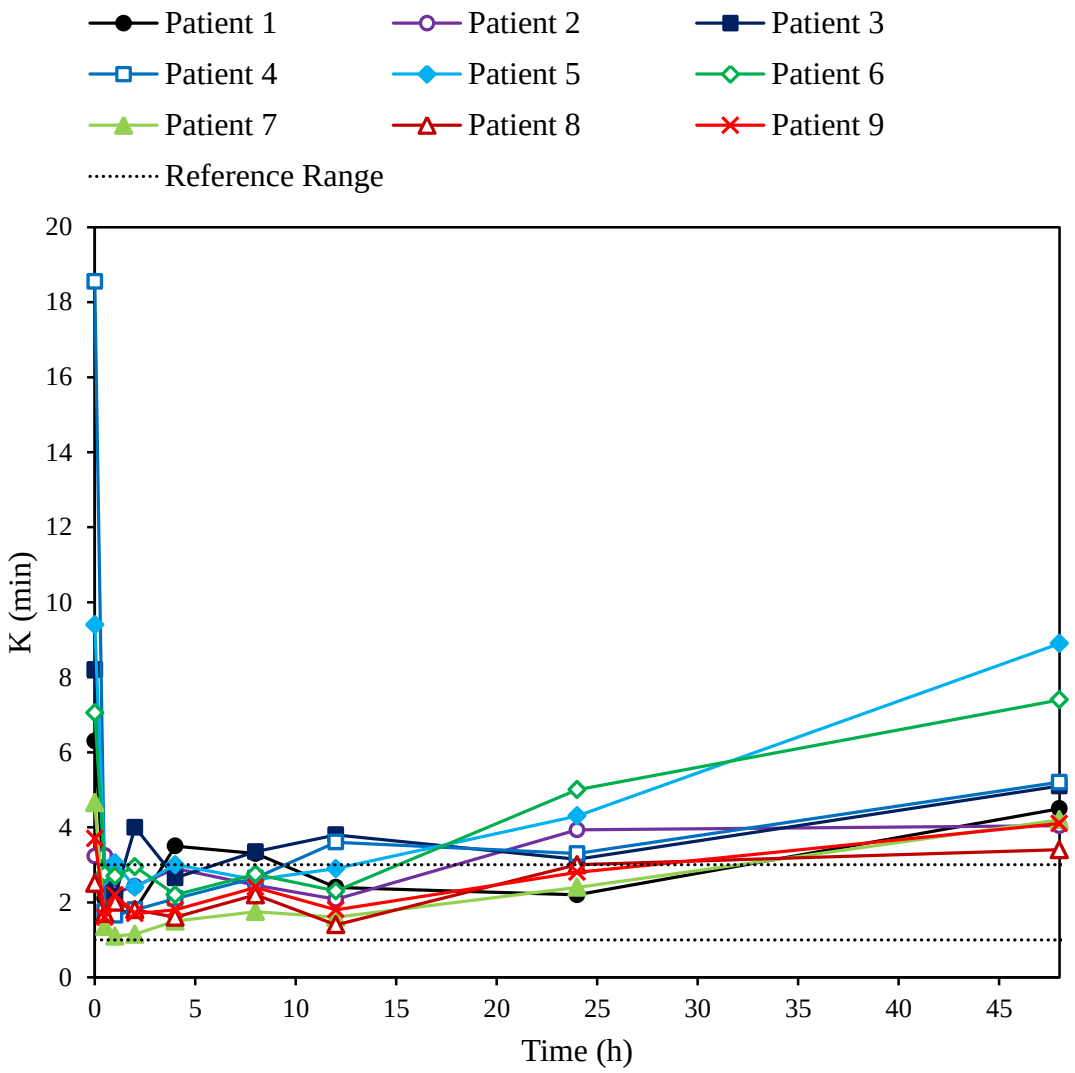
[†]Other individual profiles are presented in Appendices 5–13.

4.5.6. Kinetics-Time:

K-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.24) showed changing K over time, which is a critical prerequisite for PK/PD modeling of K. In addition, K-plasma FVIII:C profiles over 48-h following rFVIII prophylactic dosing (Figure 4.25) exhibited a clockwise hysteresis loop, i.e., as plasma FVIII:C increased, the reduction in K was delayed. Therefore, K was PK/PD modeled using the sigmoidal E_{max} model that assumes a delay in the PD response of a drug with increasing its plasma level, i.e., increasing plasma FVIII:C corresponds to a delayed reduction in K in the biophase (site of action [clot]).[75] Statistics and

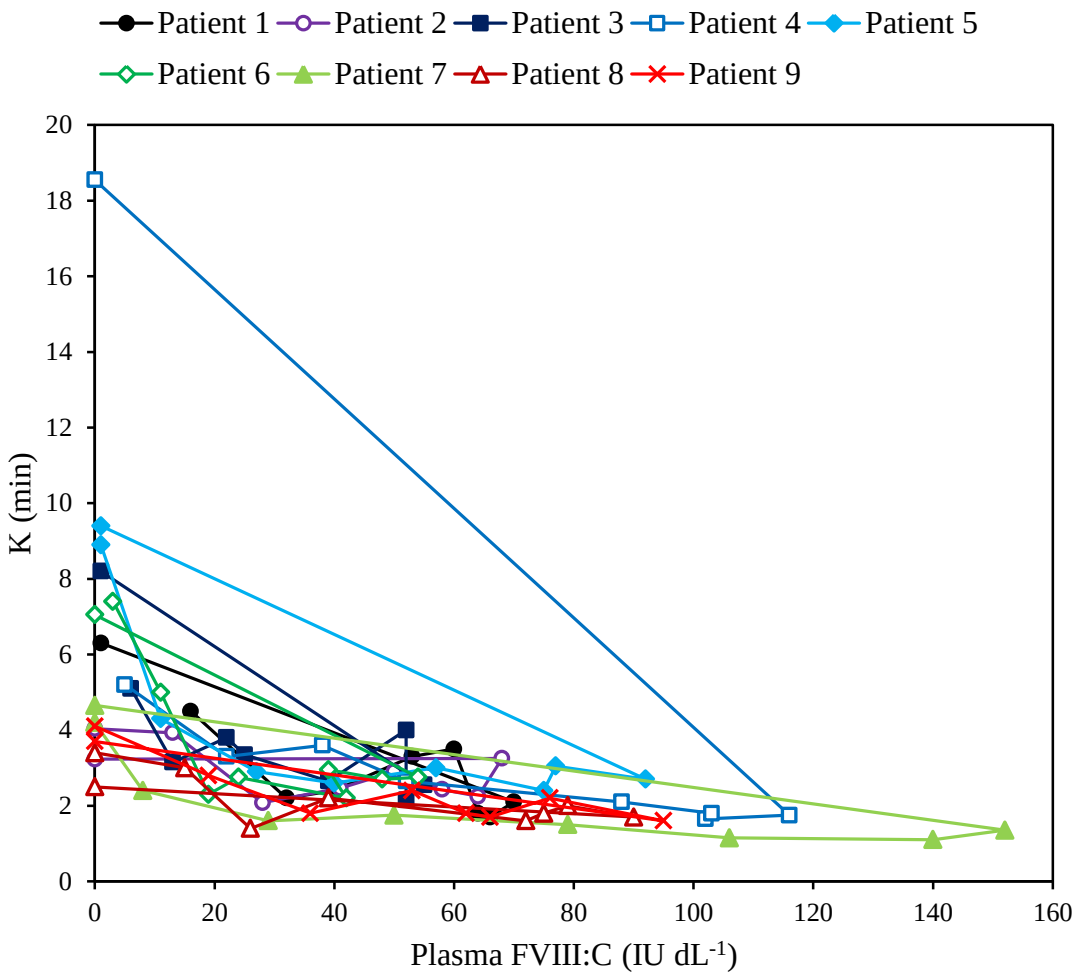
estimated PD parameters (E_0, E_{max}, EC_{50} and n [a measure of K-plasma FVIII:C curve steepness]) by fitting plasma FVIII:C dependency on K to the sigmoidal E_{max} model using non-linear regression are presented in Tables 4.21 and 4.22. The sigmoidal E_{max} model showed a good fit as indicated by r^2 (0.956–0.999). MSC (-0.46–4.47) indicated a negligible to very high degree of over-parameterization. There was a high precision in E_0 estimation as indicated by low associated SD. There was a very low to very high precision in E_{max} estimation as indicated by very low to very high associated SD. There was a very low precision in EC_{50} estimation as indicated by very high associated SD, except Patient 5 (a medium precision in EC_{50} estimation as indicated by medium associated SD). The upper limit for the EC_{50} was set as the C_{max}, which may have affected proper estimation of EC_{50}. There was a very low precision in n estimation as indicated by very high associated SD, except Patient 5 and 7 (a medium precision in n estimation as indicated by medium associated SD). PK/PD sigmoidal E_{max} modeling of K showed a delayed reduction in the corresponding predicted K with increasing the predicted plasma FVIII:C (Figures 4.26 and 4.27). The mean (±SD) E_0 was 7.3 (±4.8) min, and the median (range) E_0 was 6.6 (3.0–18.6) min. The mean (±SD) E_{max} was 6.8 (±8.7) min, i.e., the mean (±SD) E_{max} was reduced by 73.2 (±36.4) %. The median (range) E_{max} was -4.9 (-29.2– -1.1) min, i.e., the median (range) E_{max} was reduced by 71.3 (29.8–157.2) %. The mean (±SD) EC_{50} was 67.2 (±29.0) IU dL^{-1}, and the median (range) EC_{50} was 68.0 (31.4–116.0) IU dL^{-1}. The mean (±SD) n was 1.41 (±0.91), and the median (range) n was 1.83 (0.13–2.90).

Figure 4.24. K-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Figure 4.25. K-Plasma FVIII:C Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

Table 4.21. K PK/PD Sigmoidal E_{max} Model

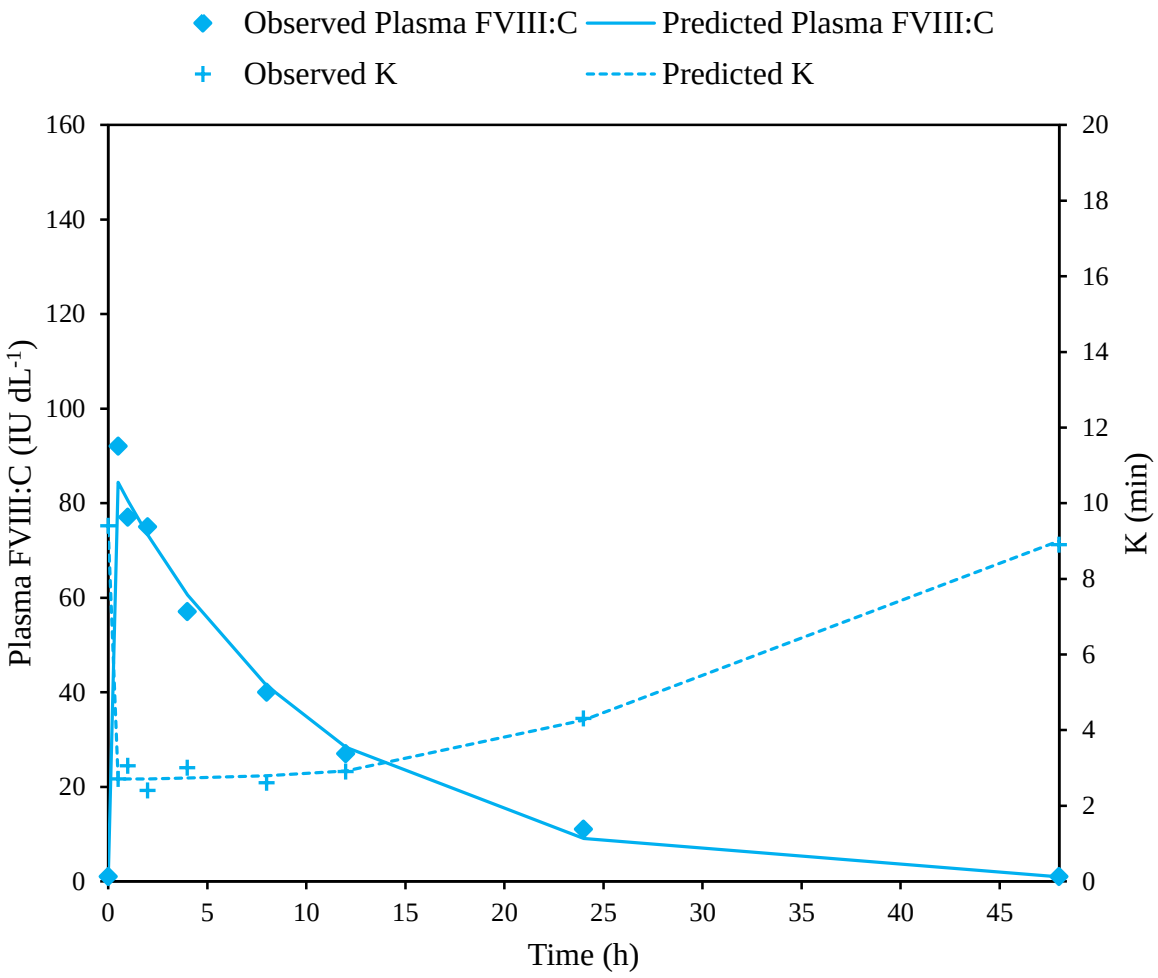
ID	r^2	MSC	E_0 (min)	(±SD)	E_{max} (min)	(±SD)
Patient 1	0.956	0.50	6.6	(±1.0)	-4.9	(±5.9)
Patient 2	0.968	-0.46	3.7	(±0.7)	-1.1	(±0.9)
Patient 3	0.983	1.40	8.2	(±0.7)	-6.9	(±6.9)
Patient 4	0.997	4.47	18.6	(±0.5)	-29.2	(±59.4)
Patient 5	0.999	4.29	9.3	(±0.3)	-6.6	(±0.3)
Patient 6	0.986	1.76	7.6	(±0.7)	-5.0	(±0.9)
Patient 7	0.997	3.54	4.7	(±0.2)	-3.8	(±0.4)
Patient 8	0.970	0.08	3.0	(±0.4)	-1.2	(±0.5)
Patient 9	0.985	1.08	4.1	(±0.4)	-2.2	(±0.6)

ID	E_{max} (% Reduction)	EC_{50} (IU dL^{-1})	(±SD)	n	(±SD)
Patient 1	74.0	70.0	(±105.8)	0.96	(±4.97)
Patient 2	29.8	68.0	(±157.6)	2.09	(±6.38)
Patient 3	84.2	31.4	(±128.3)	0.48	(±0.97)
Patient 4	157.2	116.0	(±3,608.0)	0.13	(±0.29)
Patient 5	71.3	48.7	(±9.9)	1.84	(±0.52)
Patient 6	65.9	54.0	(±24.9)	1.86	(±1.14)
Patient 7	81.2	31.7	(±15.0)	0.60	(±0.15)
Patient 8	40.2	90.0	(±94.4)	2.90	(±17.99)
Patient 9	55.0	95.0	(±59.0)	1.83	(±1.40)

Table 4.22. K PK/PD Sigmoidal E_{max} Model Summary

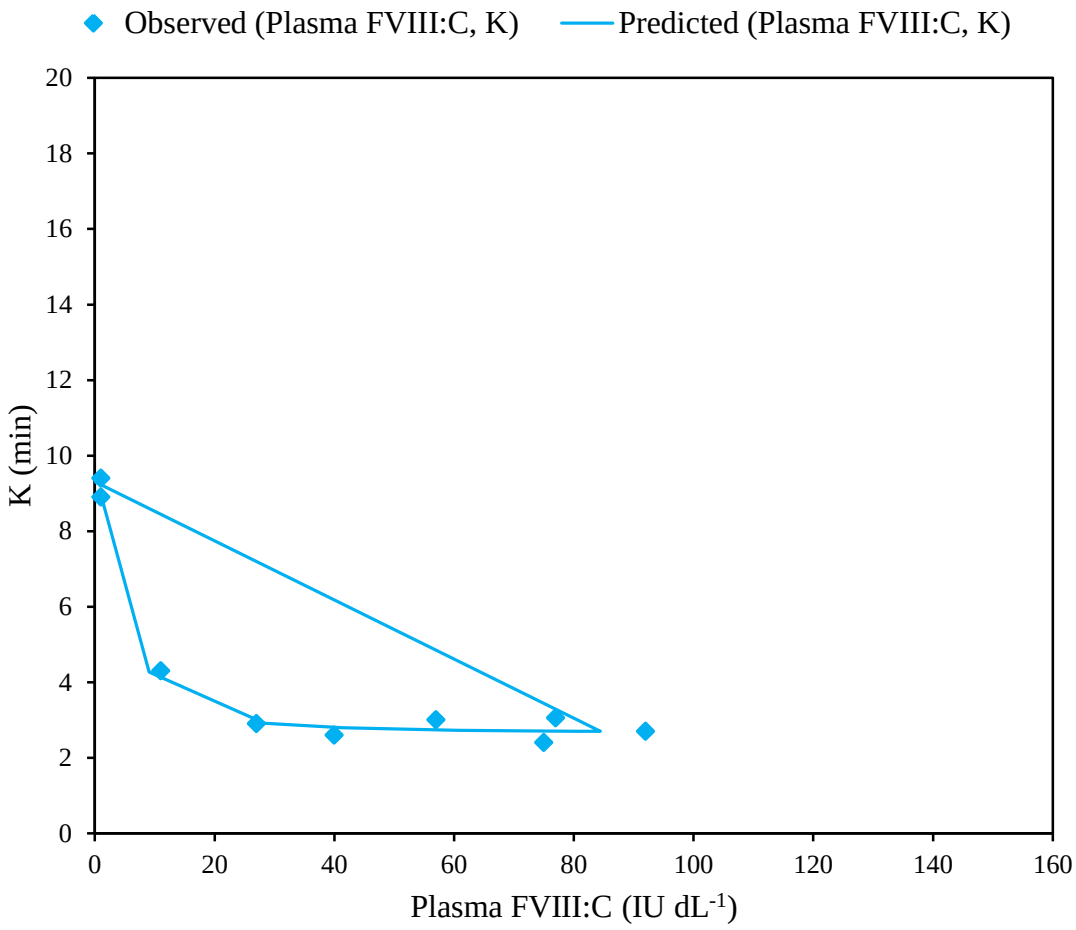
Parameter	Mean	(±SD)	Median	(Range)
E_0 (min)	7.3	(±4.8)	6.6	(3.0–18.6)
E_{max} (min)	-6.8	(±8.7)	-4.9	(-29.2– -1.1)
E_{max} (% Reduction)	73.2	(±36.4)	71.3	(29.8–157.2)
EC_{50} (IU dL^{-1})	67.2	(±29.0)	68.0	(31.4–116.0)
n	1.41	(±0.91)	1.83	(0.13–2.90)

Figure 4.26. K PK/PD Sigmoidal E_{max} Model Time Dependent Profile for Patient 5[†]



[†]Other individual profiles are presented in Appendices 5–13.

Figure 4.27. K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile for Patient 5[†]

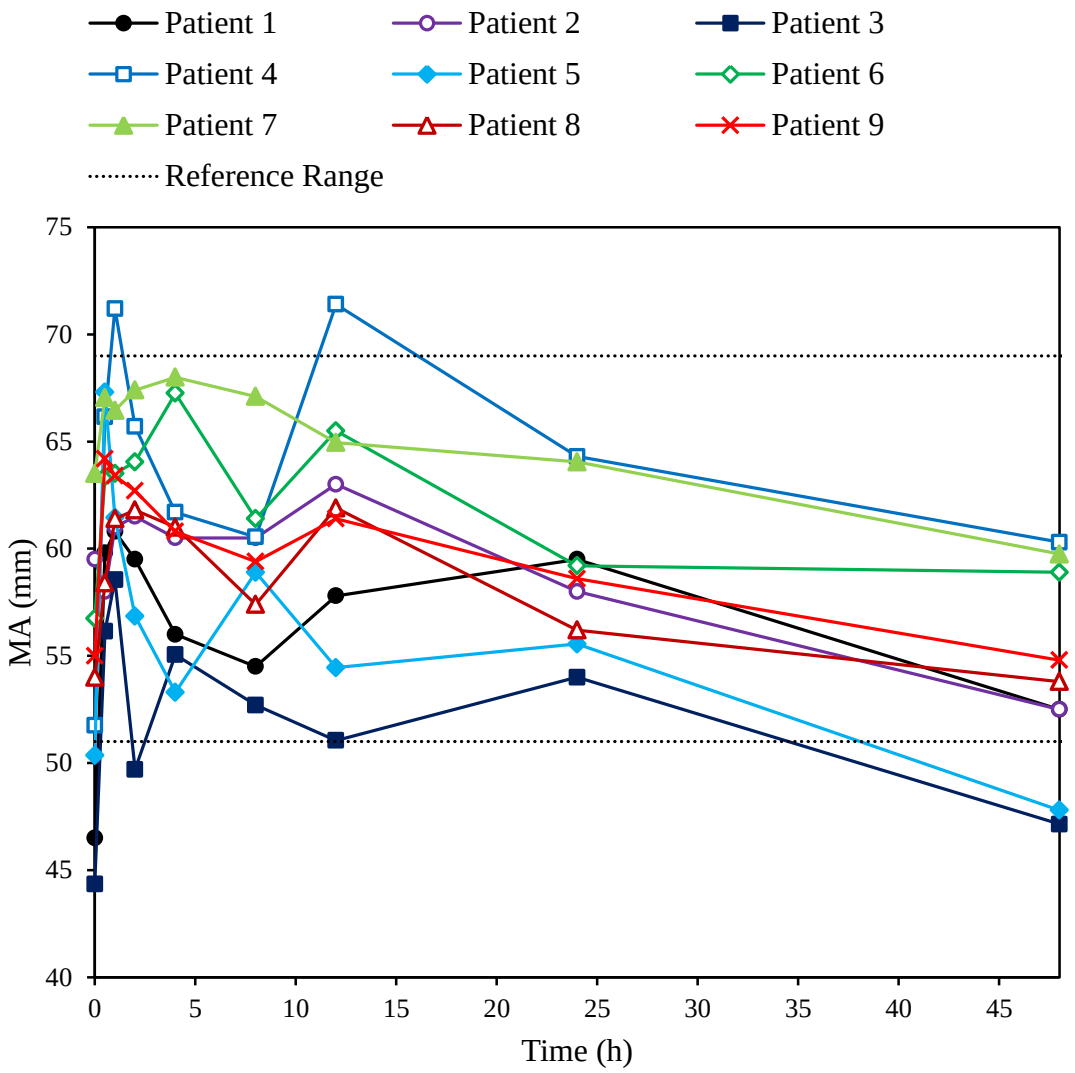


[†]Other individual profiles are presented in Appendices 5–13.

4.5.7. Maximum Amplitude:

MA-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.28) did not show changing in MA over time. MA values remained within the reference range throughout the rFVIII prophylactic dosing interval. Therefore, MA is considered a less sensitive blood viscoelastic biomarker. Changing in MA over rFVIII prophylactic dosing interval is a critical prerequisite for PK/PD modeling of MA. Therefore, MA was not PK/PD modeled.

Figure 4.28. MA-Time Profiles[†]



[†]Individual profiles are presented in Appendices 5–13.

4.6. CORRELATION AND INTER-GROUP DIFFERENCES:

Clinically relevant modifiers of bleeding tendency (plasma fibrinogen content, platelet count, age, bodyweight, ethnicity and rFVIII prophylactic regimen) were assessed, and there were no significant associations/differences regarding pertinent PK and PD parameters. There was, however, a trend in age and CL_{tot} from both NCA and CA. There were no significant inter-group differences in pertinent PK and PD parameters between frequent and infrequent bleeders.

CHAPTER 5. DISCUSSION

5.1. PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS:

To our knowledge, this was the first study that used different models to establish the PK/PD relationship of rFVIII prophylactic dosing using plasma FVIII:C and global hemostasis biomarkers (platelet function [PCF, CEM and FOT] and blood viscoelasticity [R, K and MA]) in whole blood over a 48-h prophylactic interval in 9 severe hemophiliacs (plasma FVIII:C <1 IU dL^{-1}) with variant bleeding tendency. This study also included aPTT, the widely used platelet-free biomarker in hemophilia. This study also explored the role of clinically relevant modifiers of bleeding tendency, including plasma fibrinogen content, platelet count, age, bodyweight, ethnicity and rFVIII prophylactic regimen.

5.1.1. Pharmacokinetic Modeling:

Pertinent PK parameters were estimated by both NCA and CA. NCA is an acceptable approach that provides reference PK parameters without the need to fit any data. Its accuracy and precision depend on AUC_{∞} estimation. AUC_{∞} estimation in this study was not an issue since AUC_{extra} represented a small percentage of AUC_{∞}, and therefore there was a confidence in the pertinent PK parameters (V_{ss}, CL_{tot}, $t_{1/2}$ and MRT) estimated using NCA. In addition, the pertinent PK parameters (V_{ss}, CL_{tot}, $t_{1/2}$ and MRT) estimated using NCA in this study (Table 4.8 in Chapter 4) were consistent with those reported in representative single short infusion PK studies of FVIII in hemophilia (Table 1.10 in Chapter 1).[3,35–40]

CA is more challenging since it requires fitting the data using an appropriate PK model. CA was performed using the one-compartment body model after observing a monoexponetial and a monophasic decline trend in the terminal β phase (disposition part) of plasma FVIII:C-time profiles on the linear and the logarithmic scale, respectively, which indicated instant and homogenous distribution of FVIII throughout plasma and all other tissues, making the body behaved as a one-compartment for FVIII . The one-body compartment body model is a safe approach since it requires less intensive blood sampling schedule. Overall, there was a confidence in the pertinent PK parameters (V_{ss} and CL_{tot}) estimated using the one-compartment body model as indicated by the model statistics (r^2 and MSC) and associated SD. In addition, the pertinent PK parameters (V_{ss} and CL_{tot}) estimated using the one-compartment body model (Table 4.10 in Chapter 4) were consistent with those estimated using NCA (Table 4.8 in Chapter 4) and those reported in representative single short infusion PK studies of FVIII in hemophilia (Table 1.10 in Chapter 1).[3,35–40]

5.1.2. Pharmacokinetic/Pharmacodynamic Modeling:

Changing a PD response (biomarker) over time following a drug administration is an important prerequisite for PK/PD modeling of that PD response. PD response-time profiles following rFVIII prophylactic dosing over 48-h indicated changing aPTT, PCF, CEM, FOT, R and K over time, but not MA. Therefore, aPTT, PCF, FOT, R and K were PK/PD modeled. MA (a measure of clot strength and stability) is considered to be a less sensitive blood viscoelastic biomarker because it remained within the reference range throughout the rFVIII prophylactic dosing interval. Changing aPTT over time following FVIII prophylactic dosing was consistent with the expected. Changing PCF, CEM and FOT, R, K and MA over time following FVIII prophylactic dosing have not been reported before. However, changing blood viscoelastic

biomarkers measured by Thromboelastometry (ROTEM), including clotting time (CT), clot formation time (CFT) and maximum clot firmness (MCF) over time following FVIII prophylactic dosing have been reported.[102] In general, CT, CFT and MCF are equivalent to R, K and MA, respectively. Changing R, K and MA over time in this study were consistent with changing CT, CFT and MCF, respectively, over time following FVIII prophylactic dosing as previously reported.[102]

Two PK/PD models were used: the linear and the sigmoidal E_{max}. The linear model was used for aPTT, PCF and CEM upon observing linear trend (negative for aPTT; positive for PCF and CEM) in PD response-plasma FVIII:C profiles. The linear model showed that increasing plasma FVIII:C was parallel to aPTT (a measure of the intrinsic pathway in the cascade model of blood coagulation) reduction, PCF (a measure of the force produced by platelets) increase and CEM (a measure of clot structural firmness) increase. The linear model has been used previously for aPTT to quantify FVIII replacement therapy (including its prophylaxis), especially within FVIII bioassays.[53] The linear model has not been used previously for PCF and CEM to quantify FVIII replacement therapy (including its prophylaxis). Two pertinent PD parameters were estimated using the linear model: E_0 and slope (PD response-plasma FVIII:C). Although the pertinent PD parameters (E_0 and slope) estimated using the linear model for some patients were acceptable (as indicated by the model statistics [r^2 and MSC] and associated SD), unacceptable estimation of the pertinent PD parameters (as indicated by the model statistics [r^2 and MSC] and associated SD) can be optimized by more intensive blood sampling schedule over the rFVIIII prophylactic dosing interval.

The sigmoidal E_{max} model was used for FOT, R and K upon observing a clockwise hysteresis loop in PD response-plasma FVIII:C profiles. The sigmoidal E_{max} model showed that

increasing plasma FVIII:C corresponded to a delayed reduction in FOT (a measure of clot initiation), R (a measure of clot initiation) and R (a measure of clot development). The sigmoidal E_{max} has not been used previously for FOT, R and K to quantify FVIII replacement therapy (including its prophylaxis). Four pertinent PD parameters were estimated using the sigmoidal E_{max} model: E_0, E_{max}, EC_{50} and n. Although the pertinent PD parameters (E_0, E_{max}, EC_{50} and n) estimated using the sigmoidal E_{max} model for some patients were acceptable (as indicated by the model statistics [r^2 and MSC] and associated SD), unacceptable estimation of the pertinent PD parameters (as indicated by the model statistics [r^2 and MSC] and associated SD) can be optimized by more intensive blood sampling schedule over a rFVIIII prophylactic dosing interval longer than 48-h. Over-parameterization was an issue in the PK/PD modeling using the sigmoidal E_{max} model since 4 pertinent PD parameters (E_0, E_{max}, EC_{50} and n) were estimated. The upper limit for the EC_{50} was set as the C_{max} in the PK/PD modeling, which may have affected proper estimation of EC_{50}. Patients with high rFVIII prophylactic doses should be considered in future studies in order to maximize the chances of having C_{max} more than EC_{50}.

5.2. PHARMACOKINETIC AND PHARMACODYNAMIC VARIABILITY:

5.2.1. Pharmacokinetic Variability:

There was inter-individual variation in plasma FVIII:C-time profiles over 48-h following rFVIII prophylactic dosing (Figures 4.2 and 4.3 in Chapter 4). It can be explained by variant degree of the pertinent PK parameters CL_{tot} and $t_{1/2}$. Plasma FVIII:C was used as a TDM standard to adjust the routine rFVIII prophylactic doses. Although the routine rFVIII prophylactic doses normalized plasma FVIII:C ($\geq$1 IU dL^{-1}) over the prophylactic interval (Figures 5.1 and 5.2), inter-individual variation in plasma FVIII:C failed to interpret variability

in bleeding tendency. For instance, low CL_{tot} and long $t_{1/2}$ were observed in both frequent (Patient 4) and infrequent (Patient 1) bleeders. High CL_{tot} and short $t_{1/2}$ were also observed in both frequent (Patient 7) and infrequent (Patient 2) bleeders. Plasma FVIII:C is measured within the plasma matrix, which does not reflect global hemostasis.

Figure 5.1. Mean (±SD) Plasma FVIII:C-Time Profiles

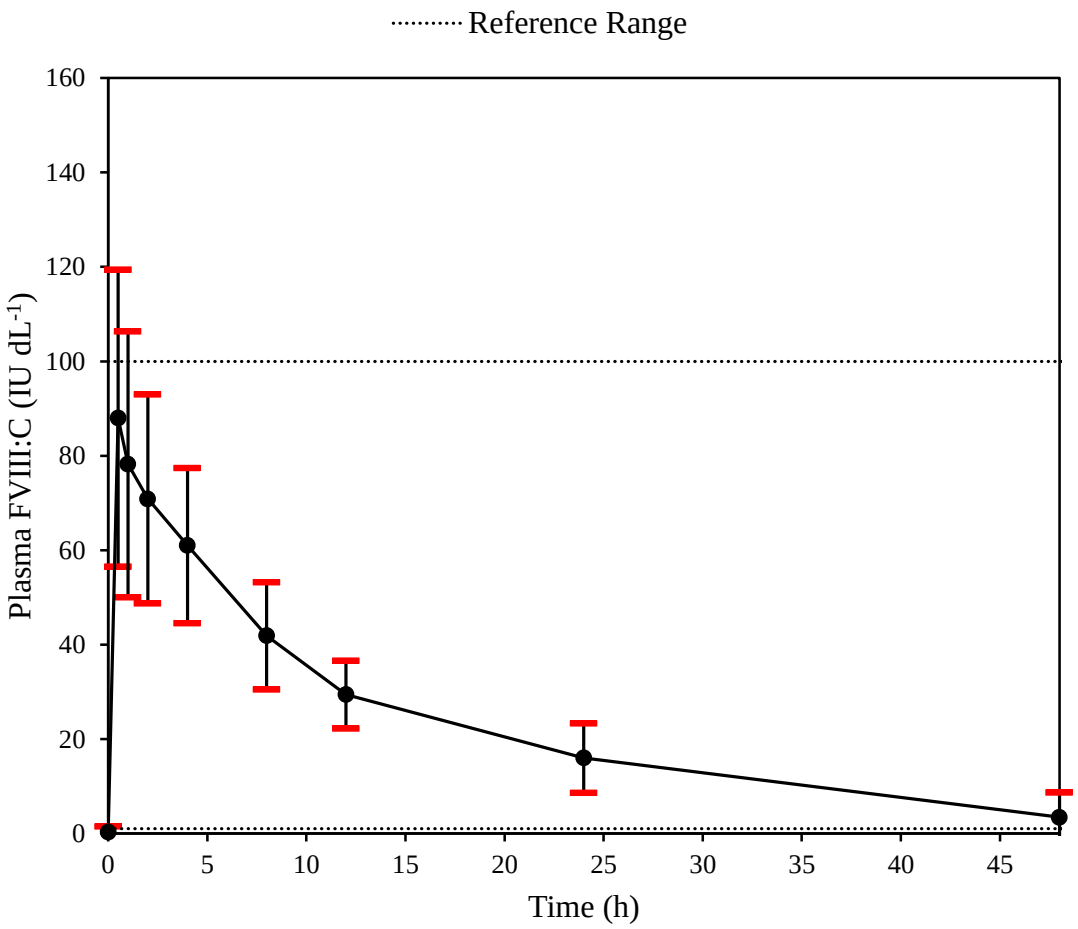
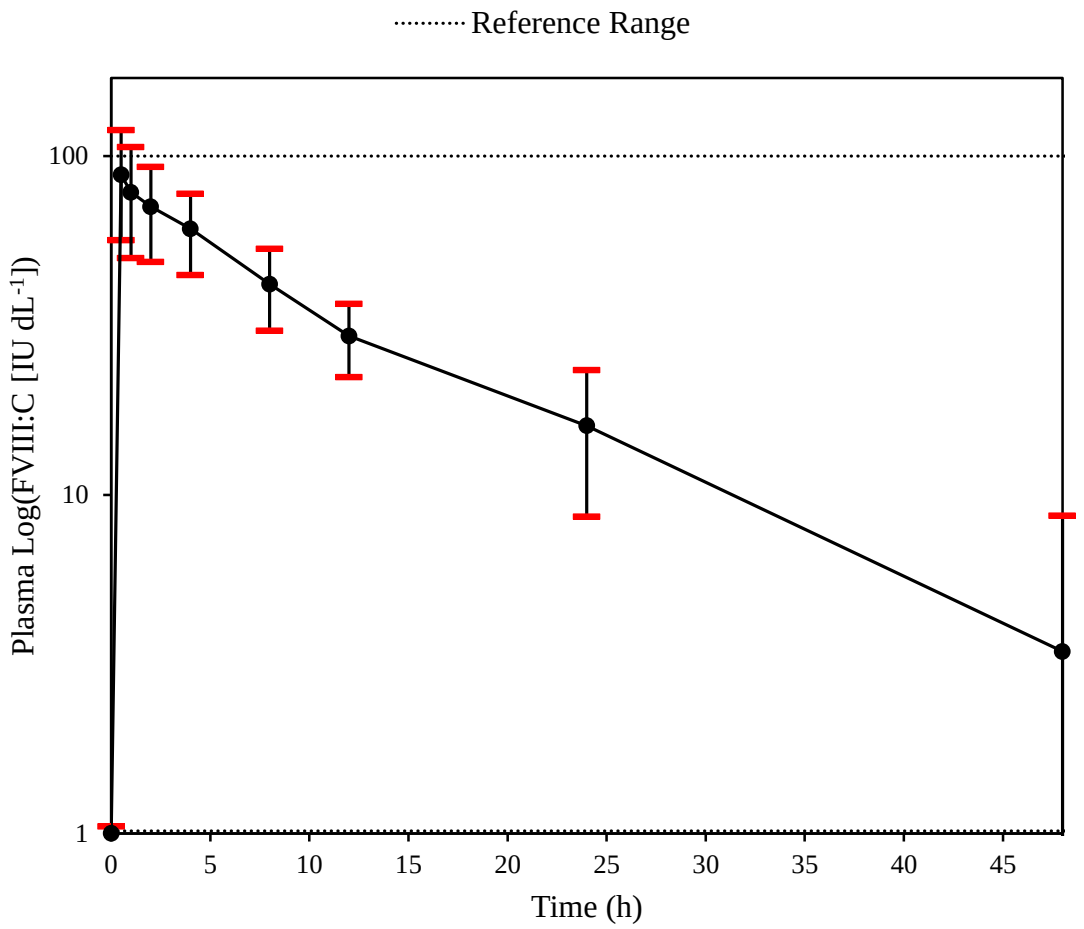


Figure 5.2. Mean (±SD) Plasma FVIII:C-Time Profiles on a Logarithmic Scale

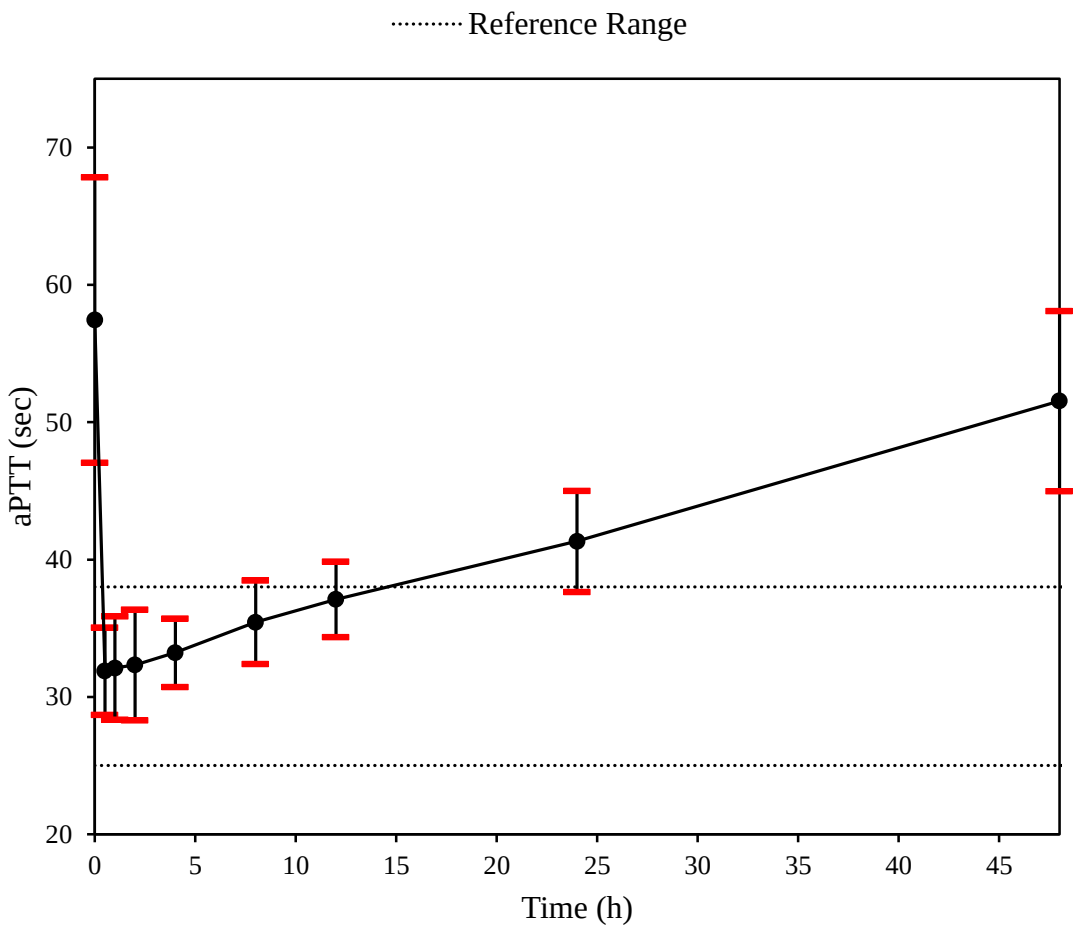


5.2.2. Pharmacodynamic Variability:

Activated Partial Thromboplastin Time. There was inter-individual variation in aPTT-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.4 in Chapter 4). For most of the patients, aPTT was sub-therapeutic by 24-h post-rFVIII prophylactic dosing (Figure 5.3). Although aPTT is an important part of FVIII bioassays, adjusting the routine rFVIII prophylactic doses by aPTT might be useful since it was not normalized throughout the entire prophylactic interval. However, the degree of inter-individual variation in aPTT was less than that of plasma FVIII:C. Consequently, variability in bleeding tendency cannot be interpreted by inter-individual

variation in aPTT. Measuring aPTT within the plasma matrix does not reflect global hemostasis, and aPTT, therefore, cannot explain viability in bleeding tendency.

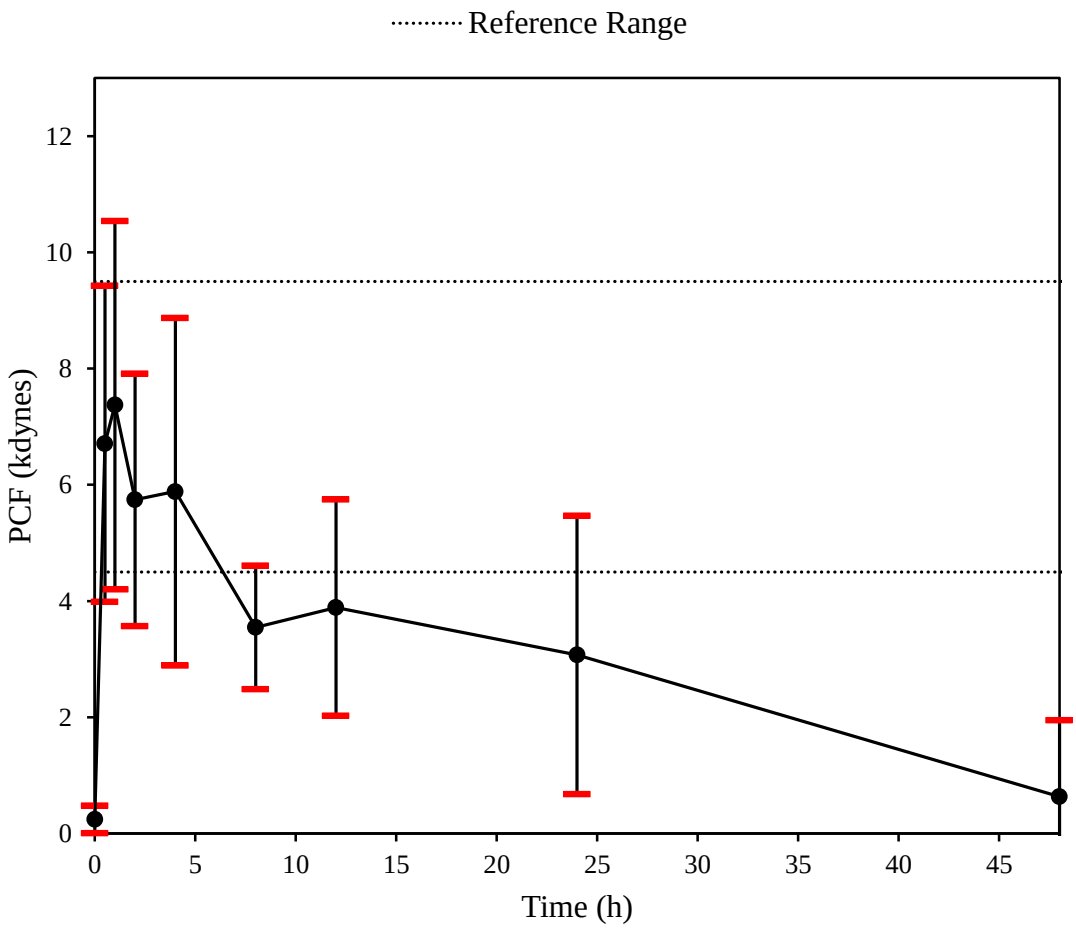
Figure 5.3. Mean (±SD) aPTT-Time Profiles



Platelet Contractile Force. There was inter-individual variation in PCF-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.8 in Chapter 4). For most of the patients, PCF was sub-therapeutic by 12-h post-rFVIII prophylactic dosing (Figure 5.4). Therefore, adjusting the routine rFVIII prophylactic doses by PCF might be more useful since it was not normalized throughout the prophylactic interval. The degree of inter-individual variation in PCF was higher than that of plasma FVIII:C. Consequently, inter-individual variation in PCF might

contribute to variability in bleeding tendency. Although it was not true for all patients, there was a trend of having a higher PCF over 24-h following rFVIII prophylactic dosing in infrequent bleeders versus frequent bleeders. PCF is measured within the whole blood matrix, and PCF, therefore, might better reflect global hemostasis.

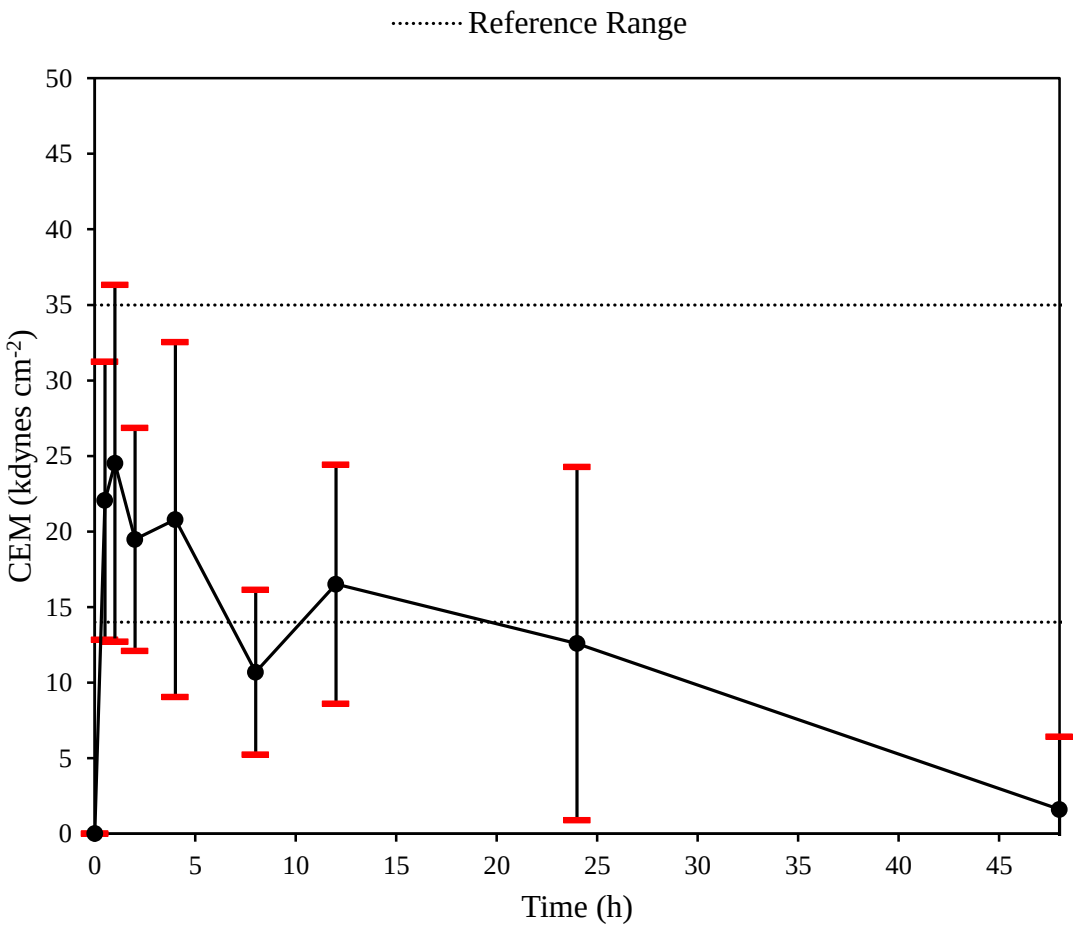
Figure 5.4. Mean (±SD) PCF-Time Profiles



Clot Elastic Modulus. There was inter-individual variation in CEM-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.12 in Chapter 4). For most of the patients, CEM was sub-therapeutic by 12-h post-rFVIII prophylactic dosing (Figure 5.5). Therefore, adjusting the routine rFVIII prophylactic doses by CEM might be more useful since it was not

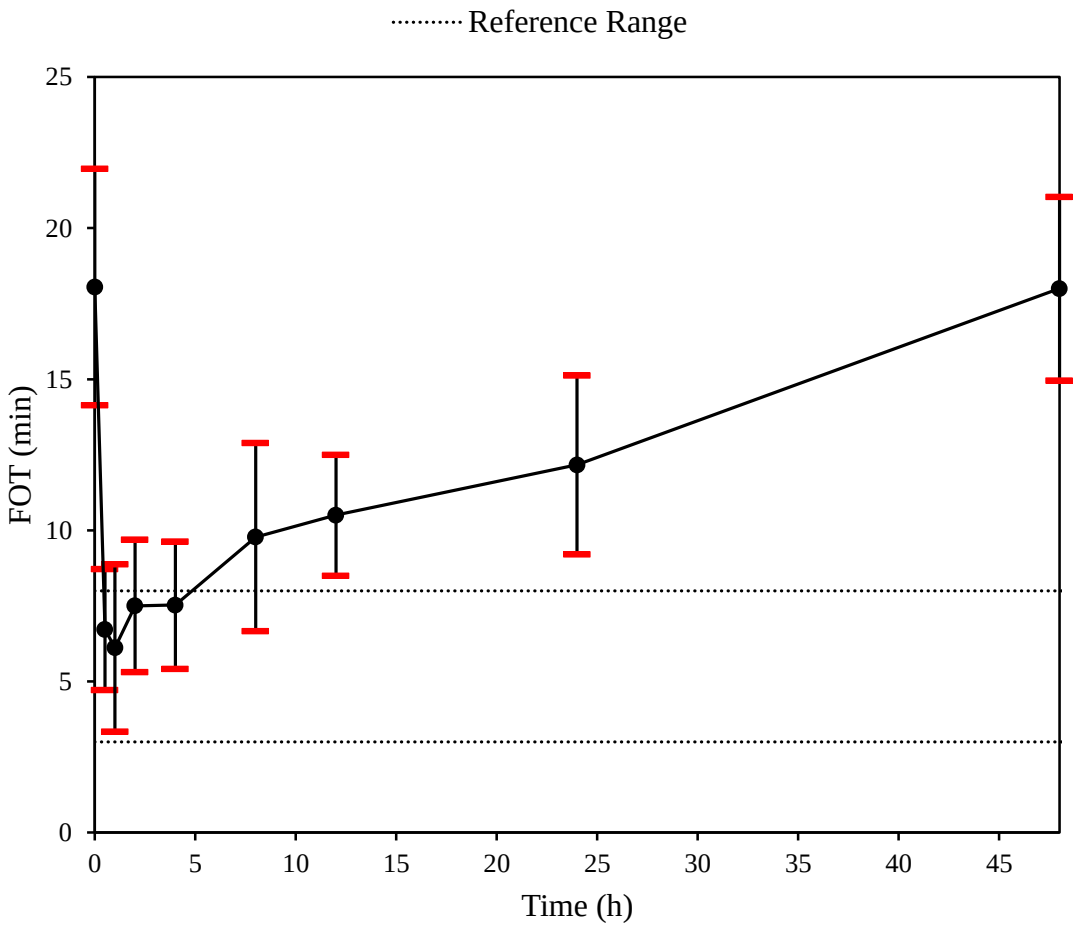
normalized throughout the prophylactic interval. The degree of inter-individual variation in CEM was higher than that of plasma FVIII:C. Consequently, inter-individual variation in CEM might contribute to variability in bleeding tendency. Although it was not true for all patients, there was a trend of having a higher CEM over 24-h following rFVIII prophylactic dosing in infrequent bleeders versus frequent bleeders. CEM is measured within the whole blood matrix, and CEM, therefore, might better reflect global hemostasis.

Figure 5.5. Mean (±SD) CEM-Time Profiles



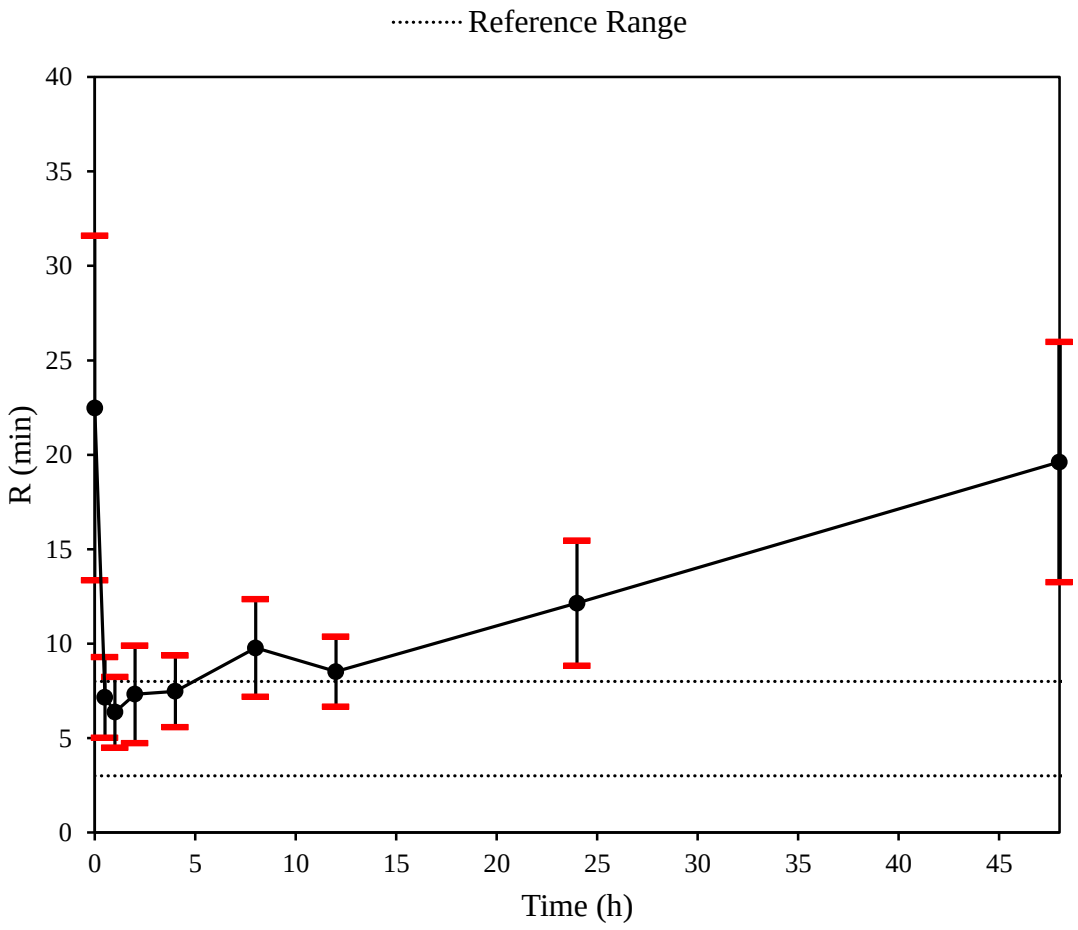
Force Onset Time. There was inter-individual variation in FOT-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.16 in Chapter 4). For most of the patients, FOT was sub-therapeutic by 8-h post-rFVIII prophylactic dosing (Figure 5.6). Therefore, FOT is more sensitive than PCF and CEM at lower plasma FVIII:C, and adjusting the routine rFVIII prophylactic doses by FOT, subsequently, might be more beneficial. The degree of inter-individual variation in FOT was equivalent to that of plasma FVIII:C. In spite of that, variability in bleeding tendency might be interpreted by inter-individual variation in FOT since it was not normalized throughout the prophylactic interval. FOT is measured within the whole blood matrix, and FOT, therefore, might better reflect global hemostasis.

Figure 5.6. Mean (±SD) FOT-Time Profiles



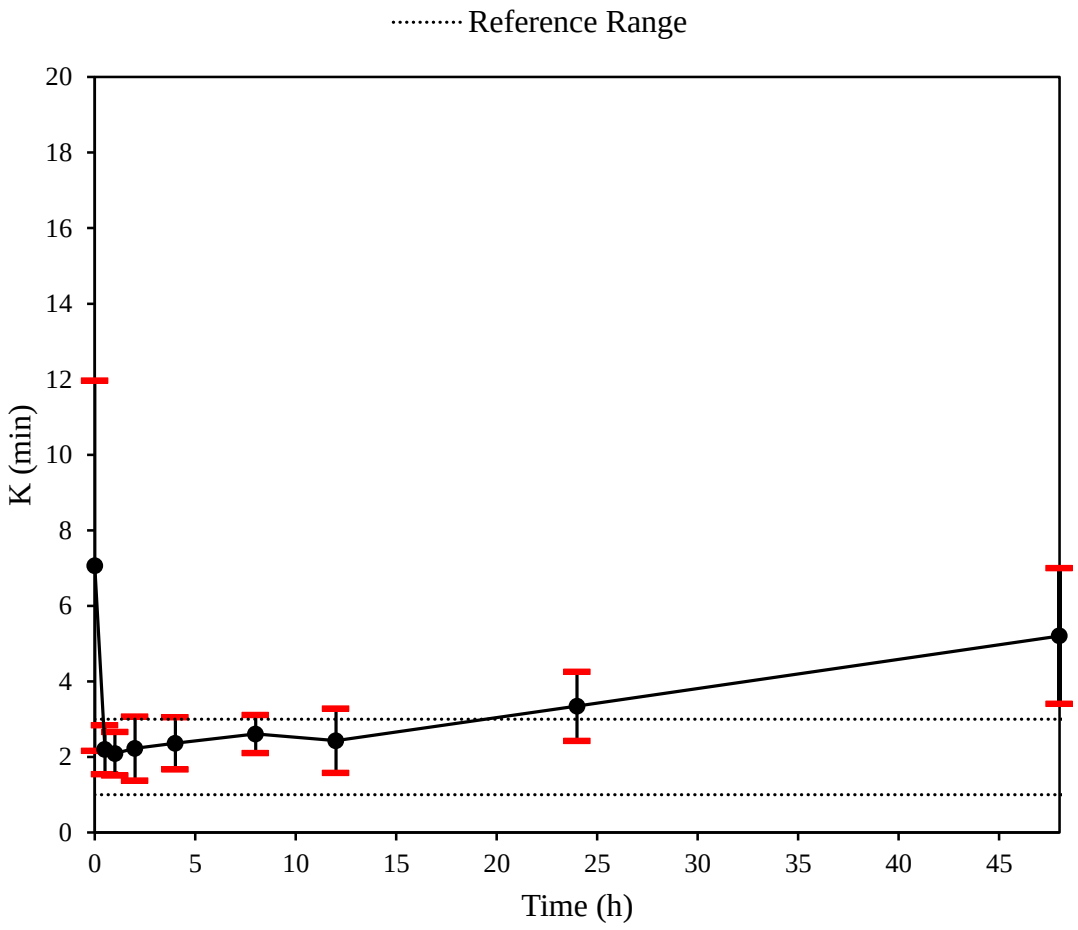
Reaction-Time. There was inter-individual variation in R-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.20 in Chapter 4). For most of the patients, R was sub-therapeutic by 8-h post-rFVIII prophylactic dosing (Figure 5.7). Therefore, R is more sensitive than PCF and CEM at lower plasma FVIII:C, and adjusting the routine rFVIII prophylactic doses by R, subsequently, might be more beneficial. The degree of inter-individual variation in R was equivalent to that of plasma FVIII:C. In spite of that, variability in bleeding tendency might be interpreted by inter-individual variation in R since it was not normalized throughout the prophylactic interval. R is measured within the whole blood matrix, and R, therefore, might better reflect global hemostasis.

Figure 5.7. Mean (±SD) R-Time Profiles



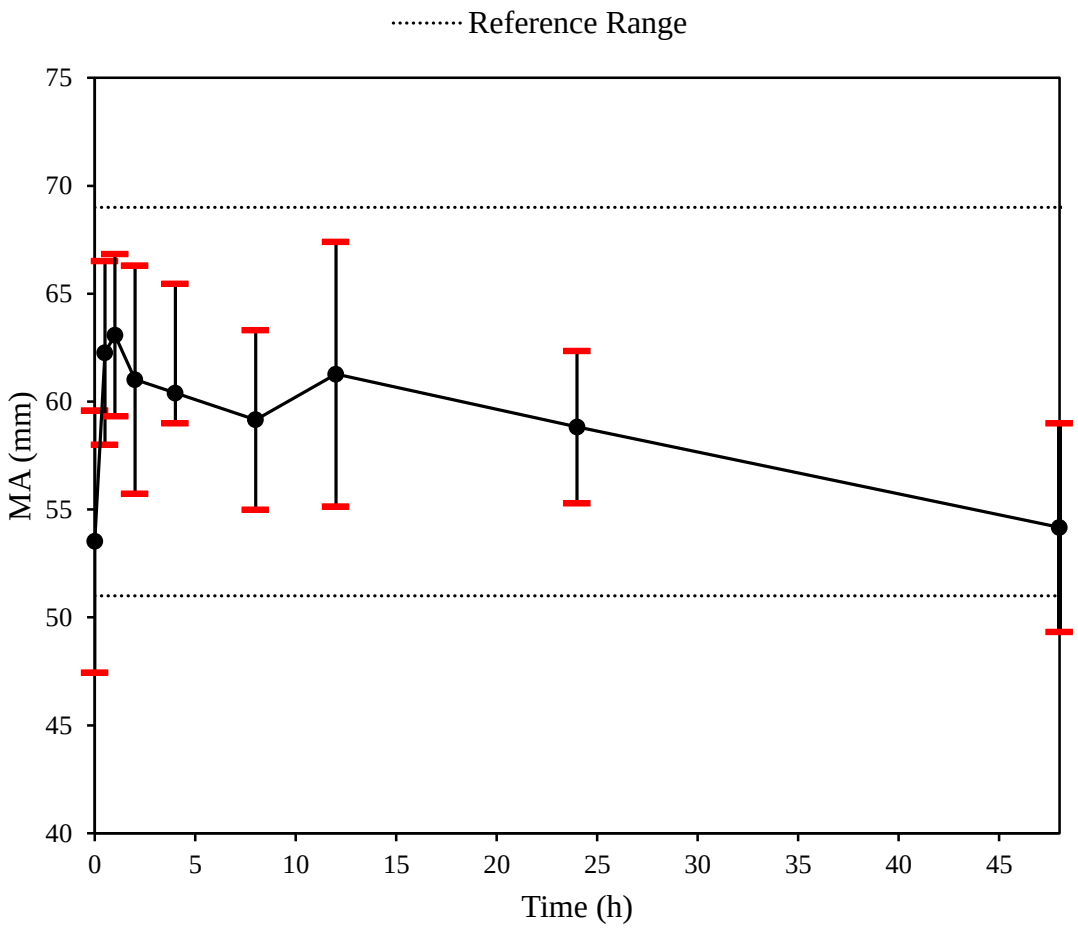
Kinetics-Time. There was inter-individual variation in K-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.24 in Chapter 4). For most of the patients, K was sub-therapeutic by 24-h post-rFVIII prophylactic dosing (Figure 5.8). Therefore, adjusting the routine rFVIII prophylactic doses by K might be more useful since it was not normalized throughout the prophylactic interval. The degree of inter-individual variation in K was equivalent to that of plasma FVIII:C. In spite of that, variability in bleeding tendency might be interpreted by inter-individual variation in K since it was not normalized throughout the prophylactic interval. K is measured within the whole blood matrix, and K, therefore, might better reflect global hemostasis.

Figure 5.8. Mean (±SD) K-Time Profiles



Maximum Amplitude. There was inter-individual variation in MA-time profiles over 48-h following rFVIII prophylactic dosing (Figure 4.28 in Chapter 4). MA was therapeutic over the entire prophylactic dosing (Figure 5.9). Therefore, MA is the least sensitive biomarker that cannot be used to adjust the routine rFVIII prophylactic doses. It was not surprising since MA depends on plasma fibrinogen content, which was within the reference range.[63] The degree of inter-individual variation in MA was less than that of plasma FVIII:C. Consequently, variability in bleeding tendency cannot be interpreted by inter-individual variation in MA.

Figure 5.9. Mean (±SD) MA-Time Profiles



5.3. LIMITATIONS:

There are a few limitations in this study. First, the sample size was small. Having a small sample size did not allow demonstrating statistical significant differences between frequent and infrequent bleeders as well as the role of clinically relevant modifiers of bleeding tendency. For instance, there was a trend between age and CL_{tot} (NCA and one-compartment body modeling) that did not reach the statistical significance. However, age was correlated with CL_{tot} in other studies with a larger sample size.[103] The small sample size of this study was a result of the budget limit as well as inadequate number of hemophiliacs that caused a slow patient recruitment. The slow patient recruitment was due to work related issues and low number of patients with severe hemophilia (plasma FVIII:C <1 IU dL^{-1}) at the site of recruitment and screening. Second, this study established the PK/PD relationship of FVIII prophylaxis in non-bleeding hemophiliacs, which was done given the larger characteristics from which to recruit. There is no logical reason to consider that the results may not be reproduced in hemophiliacs experiencing bleeding. Finally, this study did not assess the role of all clinically relevant modifiers of bleeding tendency. For instance, vWF plays an important role in FVIII CL_{tot}, and bleeding tendency may be modified according to variant vWF plasma levels.[3] In addition, coated-platelets are activated platelets that reinforce prothrombinase activity.[104] Variation in percentage of coated-platelets was connected to variability in bleeding tendency.[104] Furthermore, protein C and protein S are important contributors of fibrinolysis.[49] Variation in protein C and protein S plasma level, therefore, may contribute to variability in bleeding tendency.

5.4. CLINICAL IMPLICATIONS:

There are currently no validated TDM standards for FVIII prophylaxis to assess its hemostatic PD response in severe hemophilia. Therefore, clinicians must depend on plasma FVIII:C and clinical symptoms of resolving bleeding. Based on non-validated TDM, FVIII prophylactic dose is altered, and clinicians hope that bleeding episodes will stop. Instead, TDM tests that assess the dynamic alterations in platelet function and blood viscoelasticity may be used. Consequently, assessing risk of bleeding and individualizing FVIII prophylactic dose in more patient-specific need by observing normalized platelet function (PCF, CEM and FOT) and blood viscoelasticity (R and K) may be more beneficial in severe hemophilia.

5.5. CONCLUSIONS:

This was a pilot study that established the PK/PD relationship of rFVIII prophylactic dosing using plasma FVIII:C and global hemostasis biomarkers (platelet function [PCF, CEM and FOT] and blood viscoelasticity [R, K and MA]) as well as aPTT over a 48-h prophylactic interval in severe hemophilia. Plasma FVIII:C was PK modeled by NCA and the one-compartment body model. In addition, aPTT, PCF and CEM were PK/PD modeled by the linear model. Furthermore, FOT, R and K were PK/PD modeled by the sigmoidal E_{max} model. Plasma FVIII:C remained ≥1 IU dL^{-1} over the rFVIII prophylactic interval. Therefore, plasma FVIII:C was not a sensitive TDM standard. FOT and R, indicators of the speed of clot onset (which may relate more to the onset of bleeding in clinical settings), were more sensitive than PCF, CEM, K and aPTT since FOT and R were sub-therapeutic for a longer time at lower plasma FVIII:C following rFVIII prophylactic dosing. By applying the same concept, PCF and CEM were more sensitive than K and aPTT at lower plasma FVIII:C. MA was not PK/PD modeled because it

remained within the reference range following rFVIII prophylactic dosing. Therefore, MA was the least sensitive global hemostasis biomarker. Overall, PCF, CEM, FOT, R and K can be used to individualize FVIII prophylactic dose, monitor its hemostatic PD response and assess the risk of bleeding in severe hemophilia. However, future clinical trials should validate global hemostasis biomarkers in severe hemophilia using a larger sample size in order to demonstrate statistical significance and predict an effective FVIII prophylactic dose (and its frequency). Pilot data from this study can be used for power calculation. Multicenter clinical trials are encouraged in order to improve patient recruitment and archive the required sample size.

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List of References

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Appendices

Appendix 1. Written Informed Consent

Protocol Title: "Therapeutic Drug Monitoring of Factor VIII Prophylaxis Using Its Plasma Coagulant Activity and Global Hemostasis Biomarkers: A Pharmacokinetic/Pharmacodynamic Pilot Study"

Principle Investigator: Donald F. Brophy, PharmD, MS, FCCP, FASN, BCPS

Sponsor: The A.D. Williams Research Program

This consent form may contain words that you do not understand. Please ask the study principle investigator 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.

Objective of the Study:

Factor VIII (FVIII) is a drug that helps patients with hemophilia A, hereafter simply referred to as hemophilia, stop bleeding. However to date, there are currently no specific medical monitoring tests available to assess how well or how quickly it works in stopping bleeding. The objective of this study is to evaluate and determine the most appropriate medical monitoring tests for hemophiliacs who receive FVIII prophylaxis. You are being asked to enroll in this study because you have been diagnosed with hemophilia and may meet the study entry requirements.

Description of the Study:

FVIII prophylaxis is used to reduce bleeding episodes in hemophiliacs. Your enrollment in this study will help clinicians better understand the effects of FVIII prophylaxis in hemophiliacs. All patients who meet eligibility criteria will have blood drawn after receiving their routine recombinant factor VIII (rFVIII) prophylactic dose. Blood samples will then be tested for the clotting ability of FVIII. Your participation in this study will last up to approximately two weeks, depending on your availability. Approximately 10 patients will participate in this study. Significant new findings developed during the course of the study that may relate to your willingness to continue enrollment will be provided to you.

Procedures of the Study:

If you decide to be in this research study, you will be asked to sign this consent form after you have had all your questions answered. Your first study visit (screening visit) will be in the Central Virginia Center for Coagulation Disorders (CVCCD), which is located at the Virginia Commonwealth University (VCU) Medical Center. At your screening visit, demographics, FVIII deficiency information, rFVIII prophylaxis data, medical history (including bleeding tendency), family thrombotic history and concomitant medications will be taken. You will also receive a physical examination. A peripheral intravenous (IV) catheter will be inserted into your arm vein for blood collection. Blood, approximately two teaspoons, will be collected.

Your second study visit (inpatient visit) will take place within one week of your screening visit. At your inpatient visit, you will be admitted into the VCU General Clinical Research Center (GCRC) for 24 hours (h). Upon admission, you will receive a brief physical exam. A peripheral IV catheter will be inserted into your arm vein for rFVIII prophylactic dose

infusion and blood collection. You will be infused with your rFVIII prophylactic dose over 5 minutes (min). Blood, approximately two teaspoons, will be collected at baseline and at 0.5-, 1-, 2-, 4-, 8- and 12-h post-rFVIII prophylactic dose infusion. Before your discharge, you will receive a physical examination.

Your last study visit (outpatient visit) will take place within 24 h of your inpatient visit. Your outpatient visit will be in the CVCCD. At your outpatient visit, you will receive a brief physical examination. A peripheral IV catheter will be inserted into your arm vein for blood collection. Blood, approximately two teaspoons, will be collected. Before your release, you will receive a physical examination.

Risks and Discomforts:

Risks and discomforts of this study are minimal and relate to the process of collecting blood from you. When inserting a peripheral IV catheter into your arm vein, you may feel a slight pain, like a bee sting. Other potential risks from peripheral venous blood collection include minor bleeding, bruising, irritation of the vein, clot in the vein, infection and passing out. Risks associated with rFVIII prophylactic dosing include development of clot. Other less severe risks include fever, headache, dizziness, injection site reactions, itching, rash, hematoma, mild muscle or joint pain, swelling in joints or muscles, chest pain or tightness, difficulty breathing, nausea, vomiting, hot flashes, chills and sweating. There may be risks or adverse drug events which are unknown at this time. There is the very unlikely risk of confidentiality breach.

Benefits to You and Others:

This is not a treatment study, and you are not expected to receive any direct medical benefits from your enrollment in the study. Information from this study may lead to development

of medical monitoring tests for the clotting ability of FVIII with the hope that this new medical monitoring tests will lead to better FVIII prophylaxis in the future for patients with hemophilia.

Costs:

There are no charges for the study visits.

Payment for Enrollment:

You will be paid for your enrollment in this study if you completed all the study visits.

Alternatives:

Your alternative is not to enroll in this study.

Confidentiality:

Potentially identifiable information about you will consist of data abstracted from your medical record. Data is being collected only for the study purpose. Your data will be identified by anonymous identification (ID) number and stored separately from medical records in a secure, locked filing cabinet. All personal identifying information in electron files will be kept in a secure, password protected computer. Access to all data will be limited to study personnel. You should know that the study data about you may be reviewed or copied by the sponsor of the study or by VCU. Although results of this study may be presented at meetings or in publications, identifiable personal information pertaining to patients will not be disclosed.

Compensation for Injury:

VCU 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 enrollment in this study. If

you are injured or if you become ill as a result of your enrollment in this study, contact the study principle investigator or the study staff immediately. They 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 enrollment in this study.

Voluntary Enrollment and Withdrawal:

Your enrollment in this study is voluntary. You may decide to not enroll in this study. Your decision not to take part will involve no penalty or loss of benefits to which you are otherwise entitled. If you do enroll, you may freely withdraw from the study at any time. Your decision to withdraw will involve no penalty or loss of benefits to which you are otherwise entitled. Your enrollment in this study may be stopped at any time by the study principle investigator, the study staff or the sponsor of the study without your consent. The reasons might include: (i) it is necessary for your health or safety as might be decided by the study principle investigator or the study staff; (ii) you have not followed the study instructions; (iii) the sponsor of the study has stopped the study; or (iv) administrative reasons require your withdrawal.

Questions:

In the future, you may have questions about your study enrollment. You may also have questions about a possible adverse event to the study drug or a possible study-related injury. If you have any questions, complaints or concerns about the study, contact the study principle investigator or the VCU Office of Research. Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

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Consent:

I have been provided with an opportunity to read this written informed consent form carefully. All of the questions that I wish to raise concerning this study have been answered. By signing this written informed consent form, I have not waived any of the legal rights or benefits to which I would be otherwise entitled. My signature indicates that I freely consent to enroll in this study. I will receive a copy of the written informed consent form once I have agreed to enroll.

_____
Patient's Name (Printed)

_____ _____
Patient's Signature Date (MM/DD/YYYY)

_____
Name of Person Conducting
Written Informed Consent (Printed)

_____ _____
Signature of Person Conducting Written Informed Consent Date (MM/DD/YYYY)

_____ _____
Principle Investigator's Signature Date (MM/DD/YYYY)

Appendix 2. Screening Visit Form

Protocol Title: "Therapeutic Drug Monitoring of Factor VIII Prophylaxis Using Its Plasma Coagulant Activity and Global Hemostasis Biomarkers: A Pharmacokinetic /Pharmacodynamic Pilot Study"

Date: _____ (MM/DD/YYYY)

Patient's Identification (ID) Number: _____

Age: _____(Years)

Bodyweight: _____ (Kilogram [kg])

Ethnicity: □ African American □ American Indian or Alaska Native

□ Asian □ Caucasian

□ Hispanic □ Native Hawaiian or Other Pacific Islander

Primary Diagnosis: Severe Factor VIII (FVIII) Deficiency

Recombinant Factor VIII (rFVIII) Product (Primary Medication):

□ Advate □ Helixate FS □ Kogenate FS

□ Recombinate □ ReFacto □ Xyntha

rFVIII Prophylactic Dose: _____ (International Unit [IU])

rFVIII Prophylactic Equivalent Dose: _____(International Unit per Kilogram [IU kg^{-1}])

rFVIII Prophylactic Regimen: □ Two Times Weekly □ Three Times Weekly

Bleeding Tendency in the Past 6 Months: □ >3 Bleeding Episodes □ ≤3 Bleeding Episodes

Secondary Diagnosis:

1. _____ 2. _____

3. _____ 4. _____

5. _____ 6. _____

7. _____ 8. _____

Secondary Medications:

1. _____ 2. _____

3. _____ 4. _____

5. _____ 6. _____

7. _____ 8. _____

Data Collector's Signature: _____ **Date:** _____ (MM/DD/YYY)

Appendix 3. Inpatient Visit Form

Protocol Title: "Therapeutic Drug Monitoring of Factor VIII Prophylaxis Using Its Plasma Coagulant Activity and Global Hemostasis Biomarkers: A Pharmacokinetic /Pharmacodynamic Pilot Study"

Date: _____ (MM/DD/YYYY)

Patient's Identification (ID) Number: _____

Bodyweight: _____ (Kilogram [kg])

Recombinant Factor VIII (rFVIII) Product:

□ Advate □ Helixate FS □ Kogenate FS

□ Recombinate □ ReFacto □ Xyntha

rFVIII Product Expiration Date: _____ (MM/DD/YYYY)

rFVIII Product Lot Number: _____

rFVIII Prophylactic Dose: _____ (International Unit [IU])

rFVIII Prophylactic Equivalent Dose: _____(International Unit per Kilogram [IU kg^{-1}])

Relative Time (Hour [h])	**Actual Time** (h)	**Blood Sampling Tubes**	**Comments**
0		2	
[redacted]		[redacted]	Infuse rFVIII prophylactic dose
0.5		2	
1		2	
2		2	
4		2	
8		2	
12		2	
24		2	

Nurse's Signature: _____ **Date:** _____ (MM/DD/YYY)

Appendix 4. Outpatient Visit Form

Protocol Title: "Therapeutic Drug Monitoring of Factor VIII Prophylaxis Using Its Plasma Coagulant Activity and Global Hemostasis Biomarkers: A Pharmacokinetic /Pharmacodynamic Pilot Study"

Date: _____ (MM/DD/YYYY)

Patient's Identification (ID) Number: _____

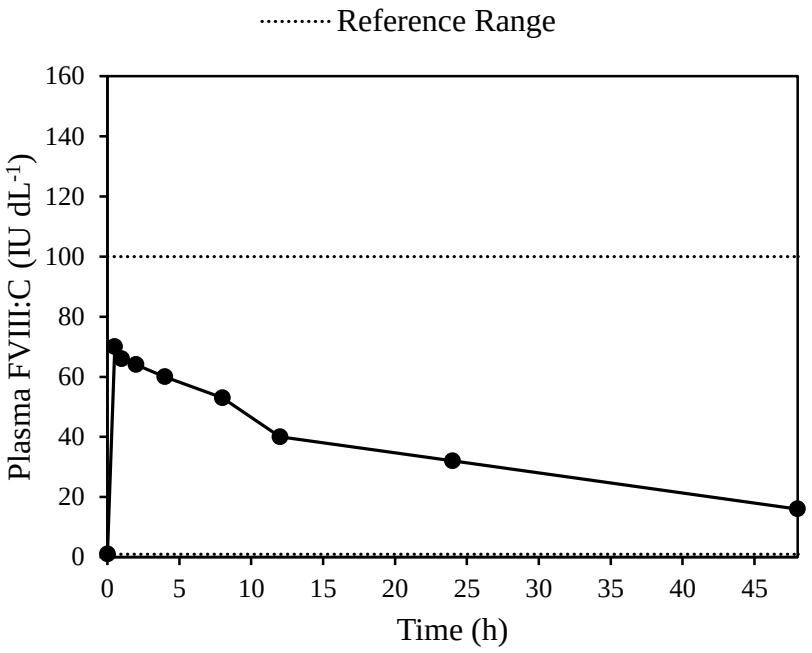
Relative Time (Hour [h])	**Actual Time** (h)	**Blood Sampling Tubes**	**Comments**
48[†]		2	

[†]Post-recombinant Factor VIII (rFVIII) prophylactic dose infusion

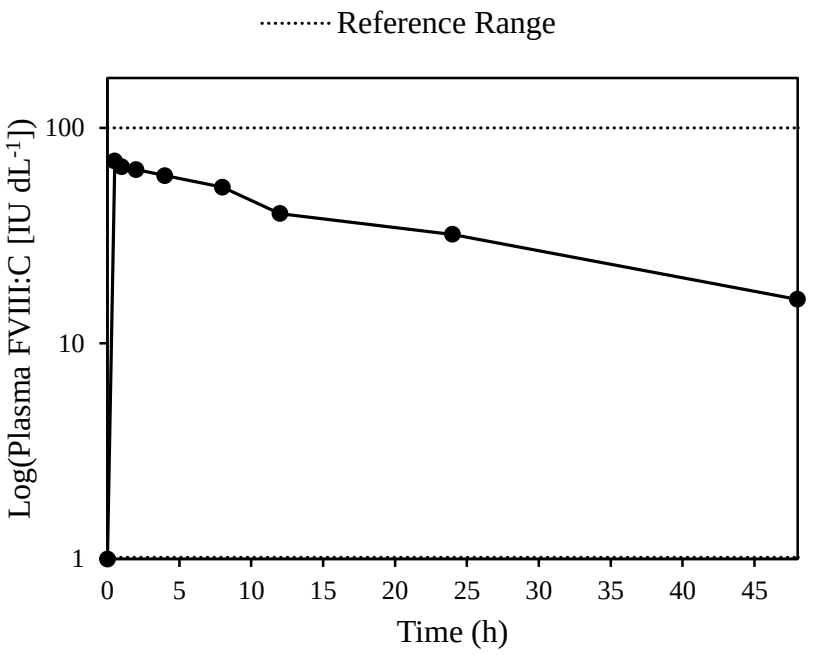
Nurse's Signature: _____ **Date:** _____ (MM/DD/YYY)

Appendix 5. Individual Profiles for Patient 1

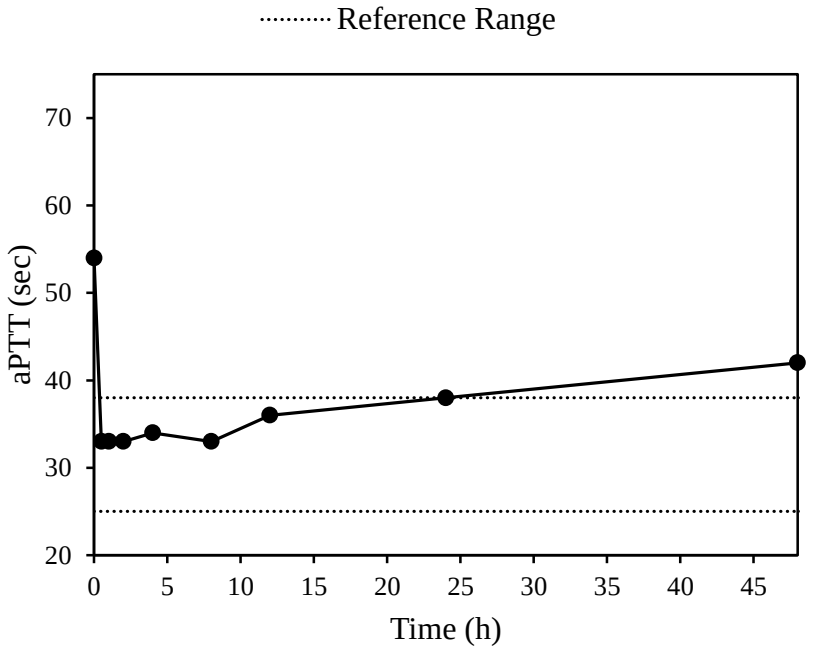
Plasma FVIII:C-Time Profiles



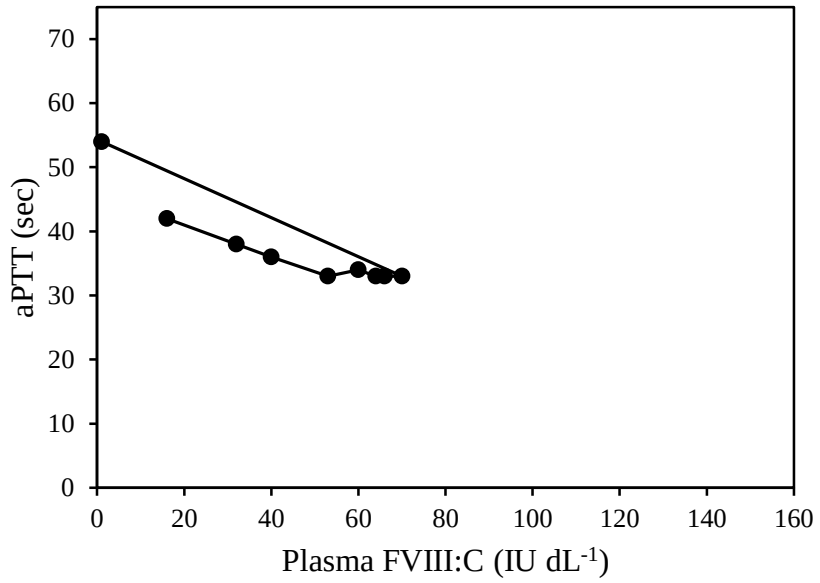
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



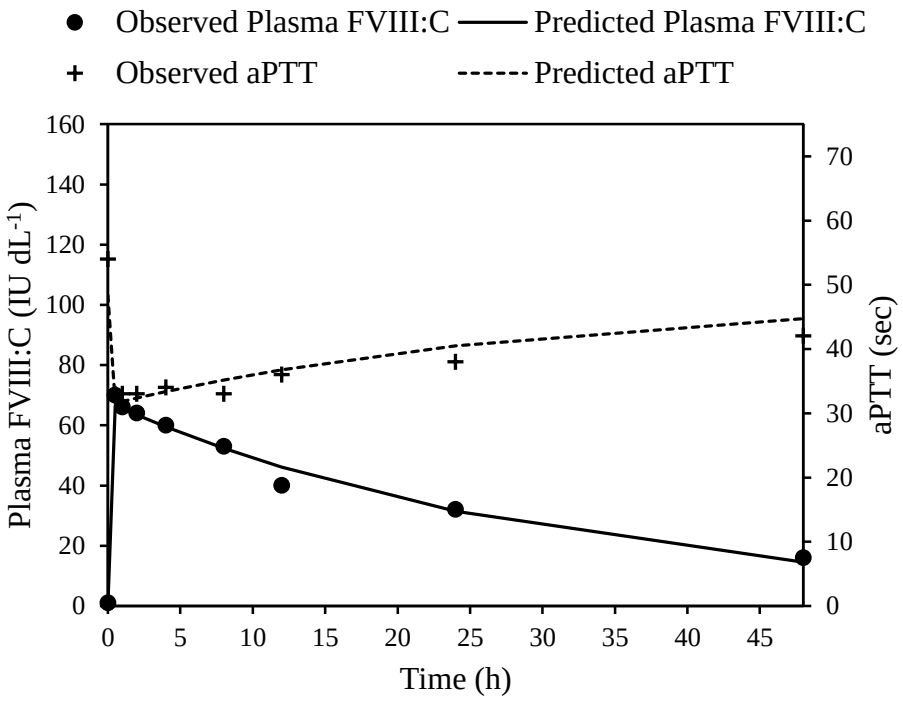
aPTT-Time Profile



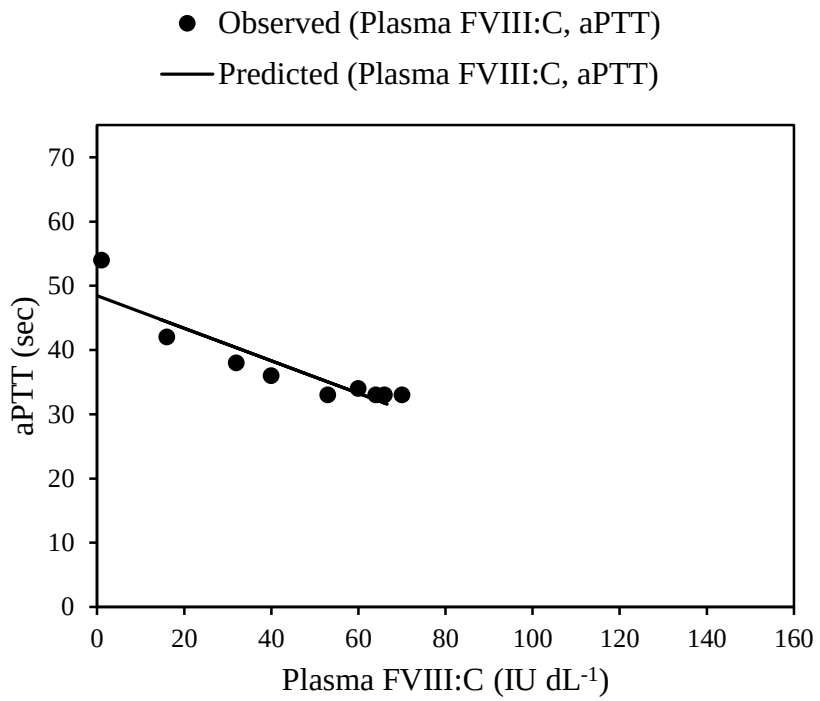
aPTT-Plasma FVIII:C Profile



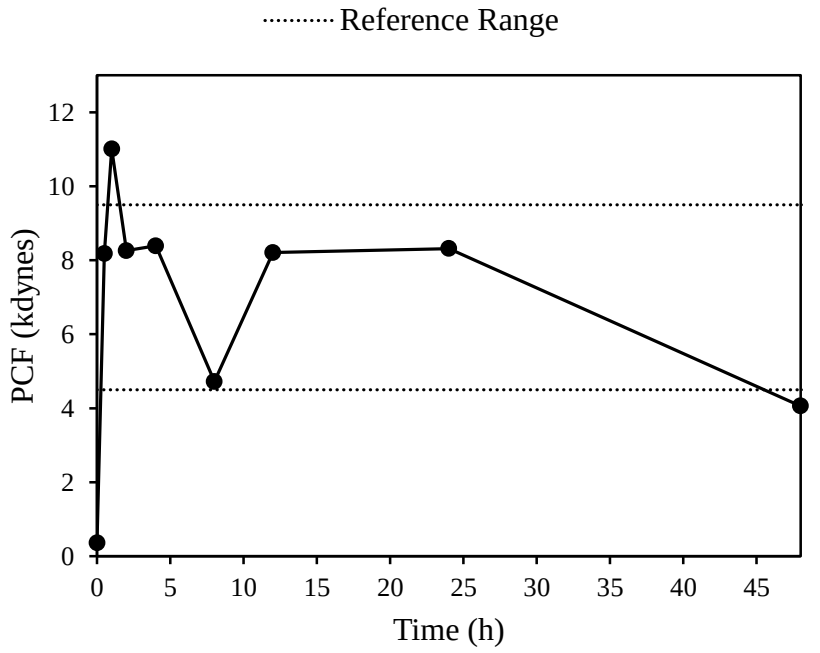
aPTT PK/PD Linear Model Time Dependent Profile



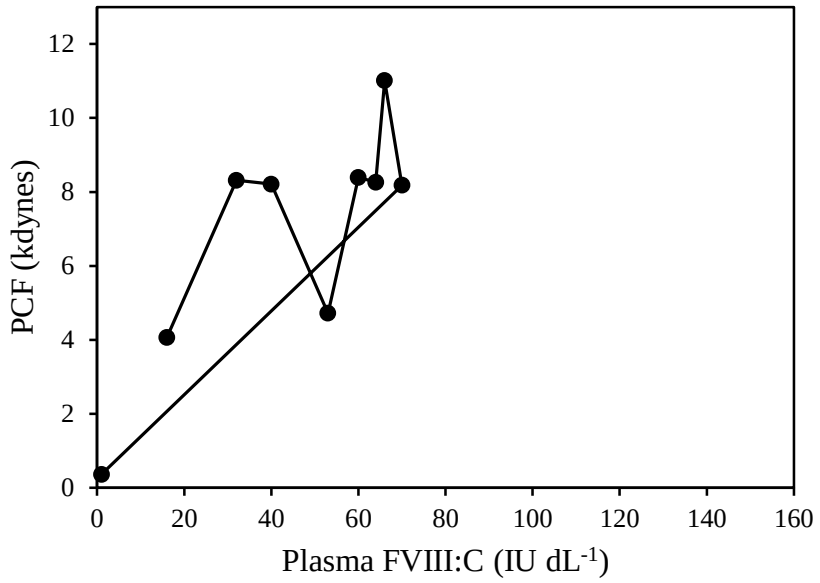
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



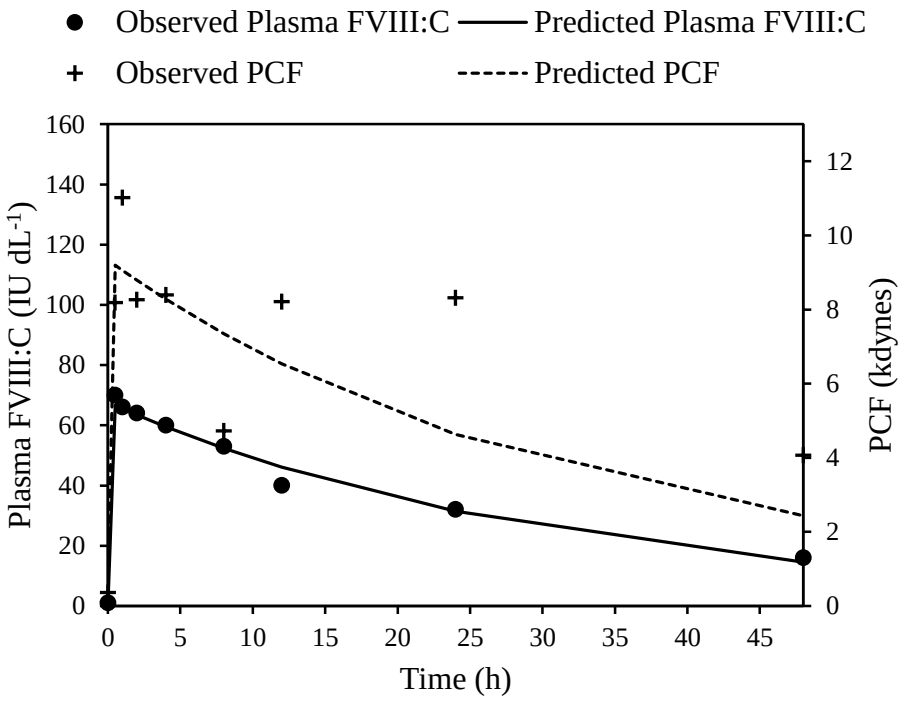
PCF-Time Profile



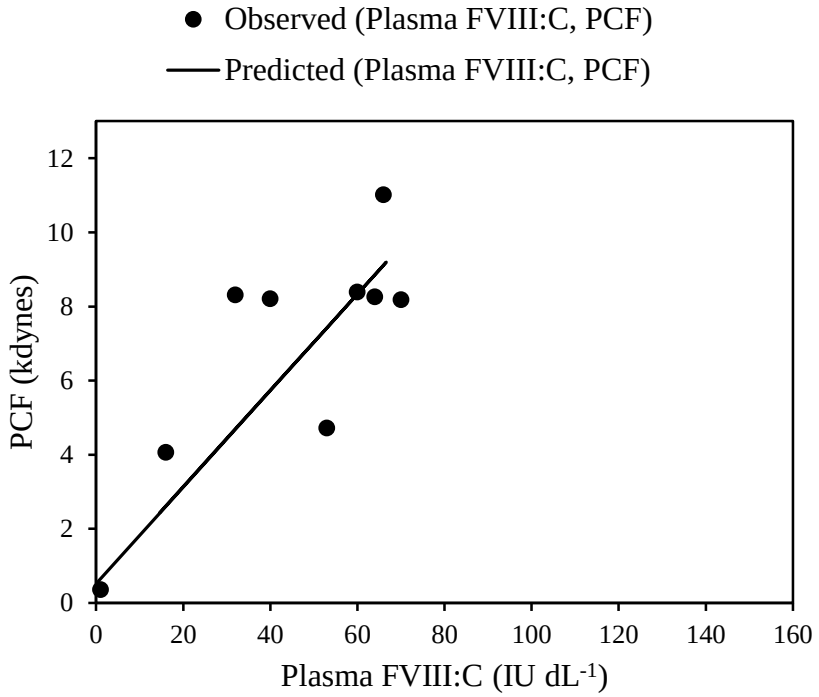
PCF-Plasma FVIII:C Profile



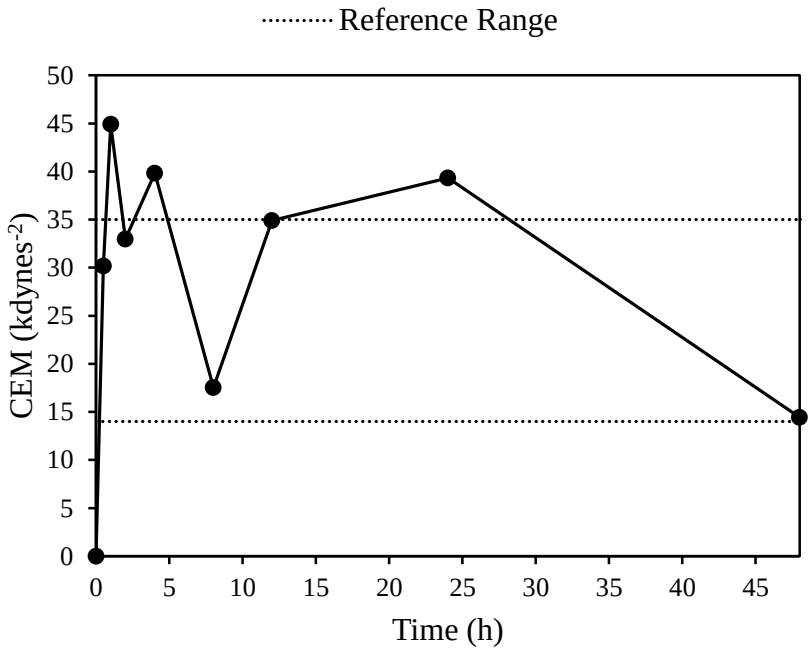
PCF PK/PD Linear Model Time Dependent Profile



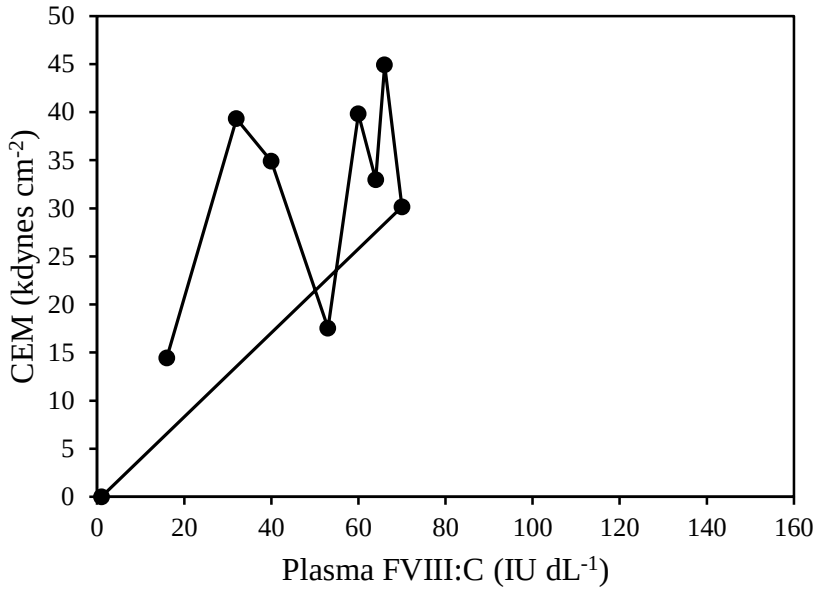
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



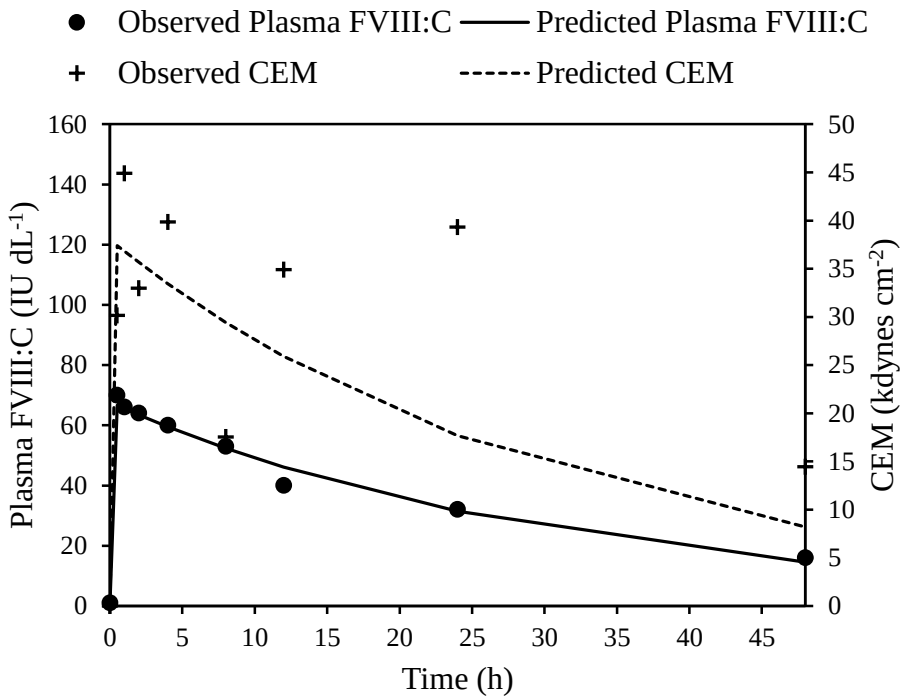
CEM-Time Profile



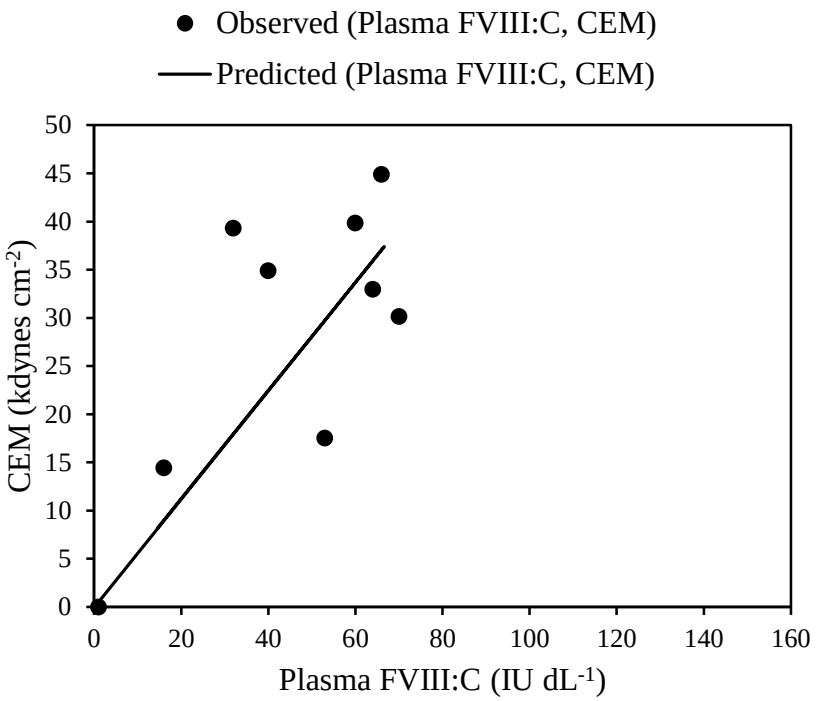
CEM-Plasma FVIII:C Profile



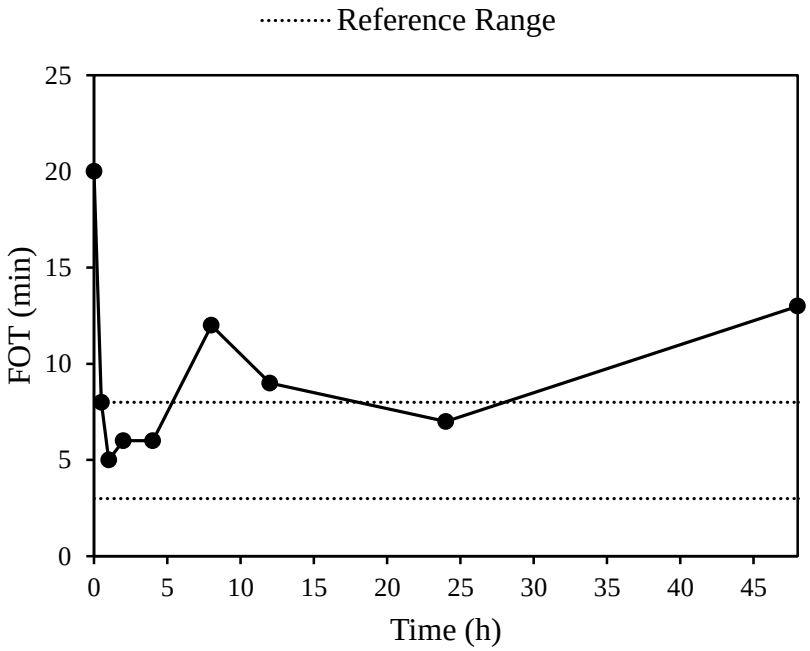
CEM PK/PD Linear Model Time Dependent Profile



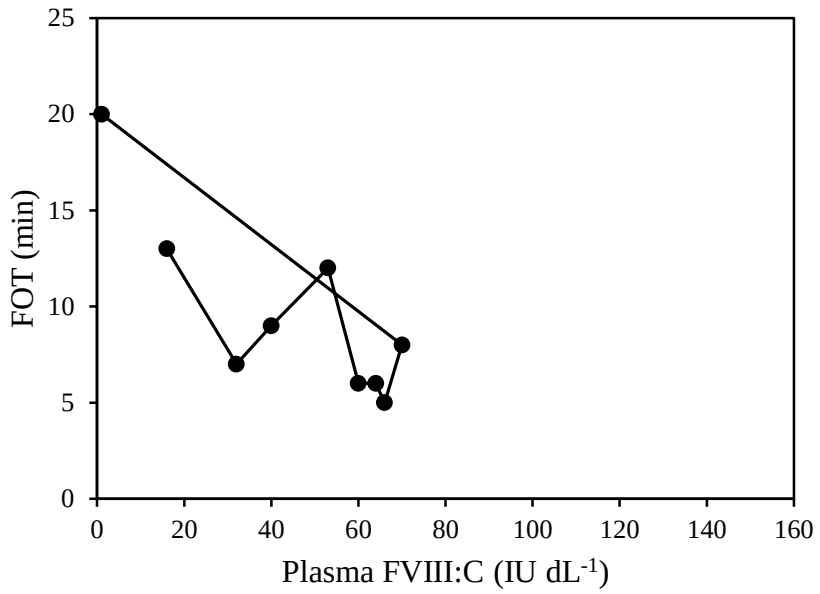
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



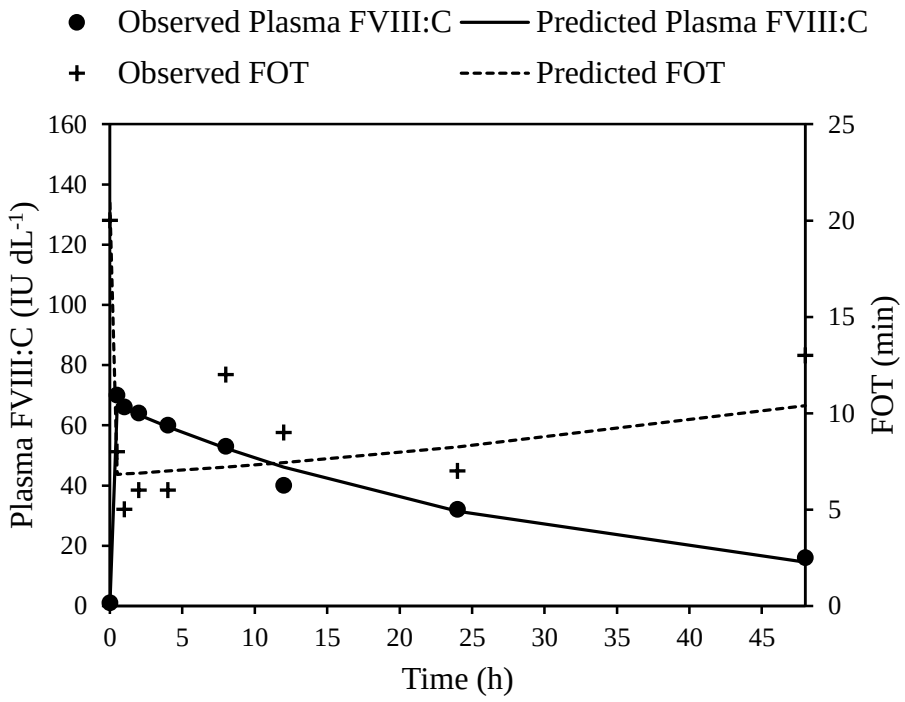
FOT-Time Profile



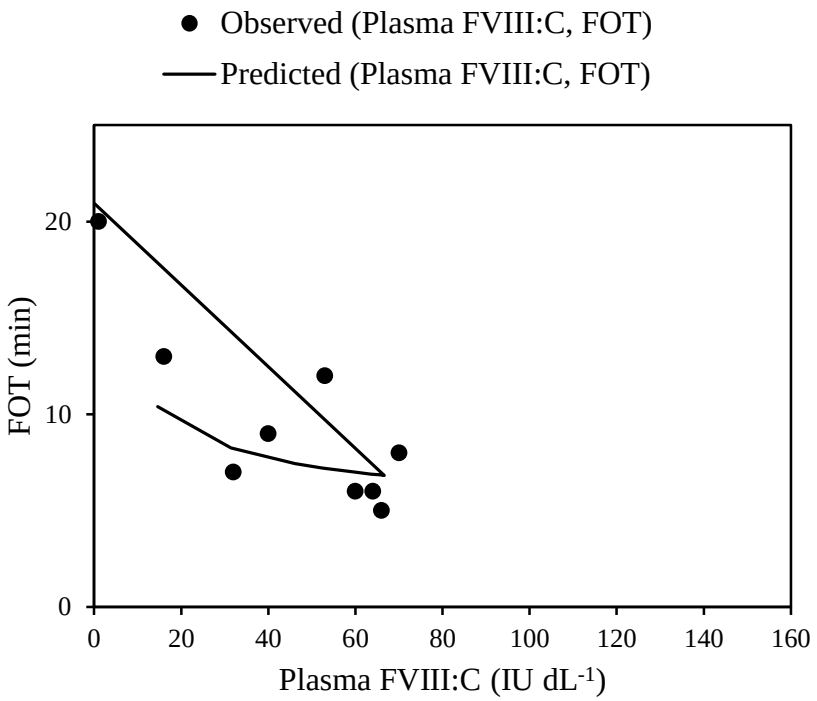
FOT-Plasma FVIII:C Profile



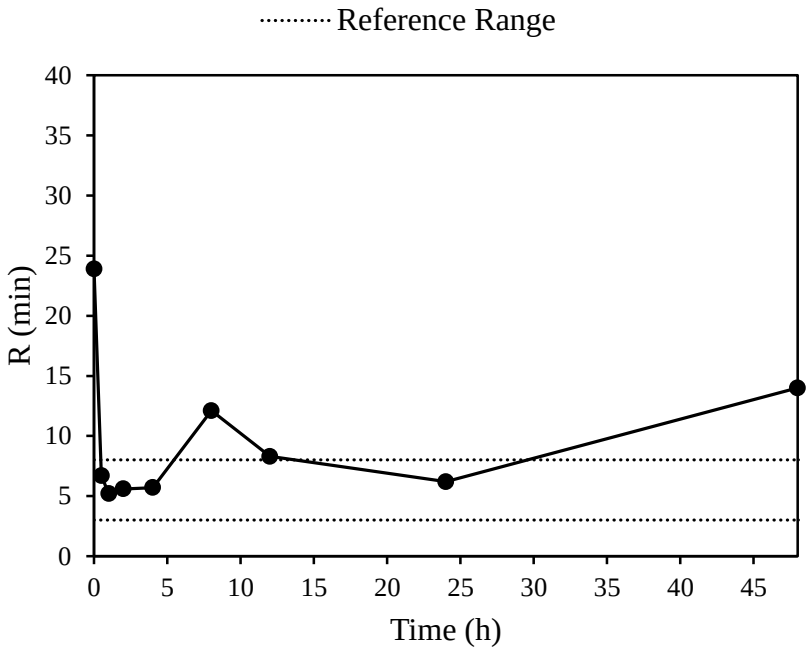
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



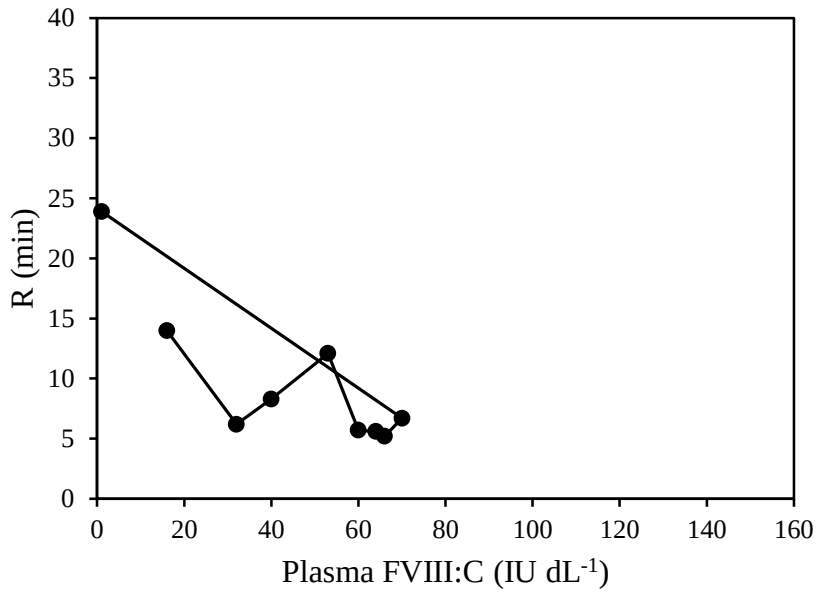
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



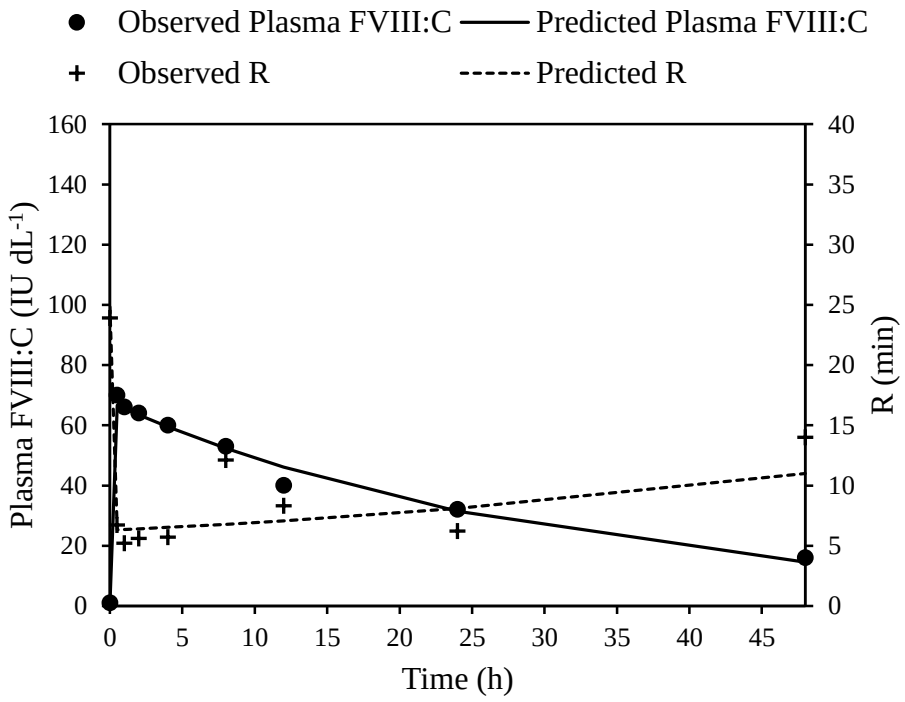
R-Time Profile



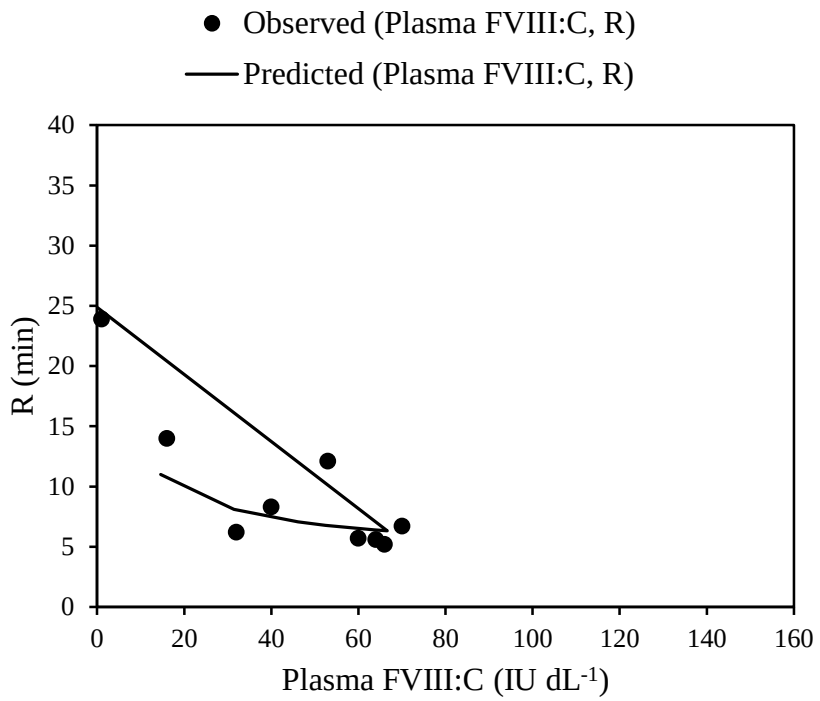
R-Plasma FVIII:C Profile



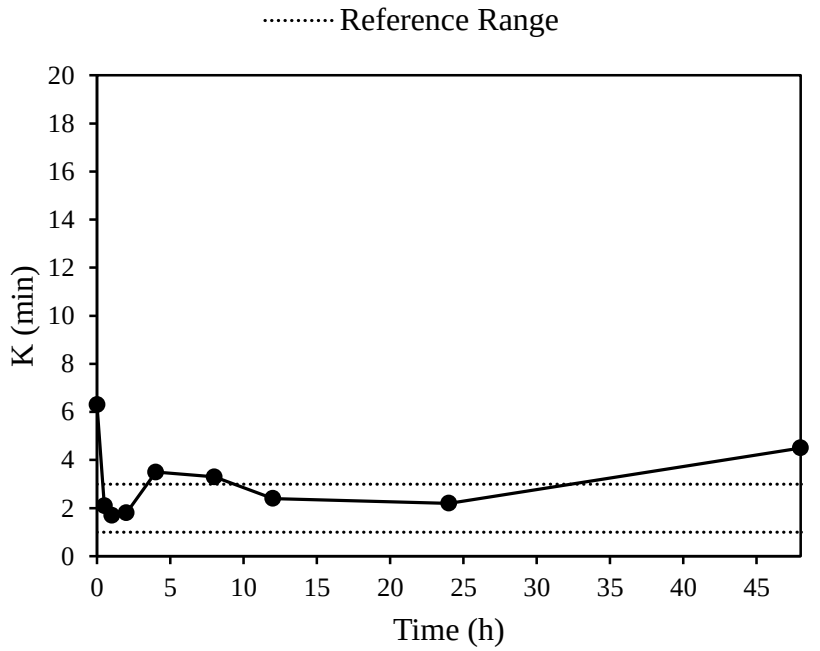
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



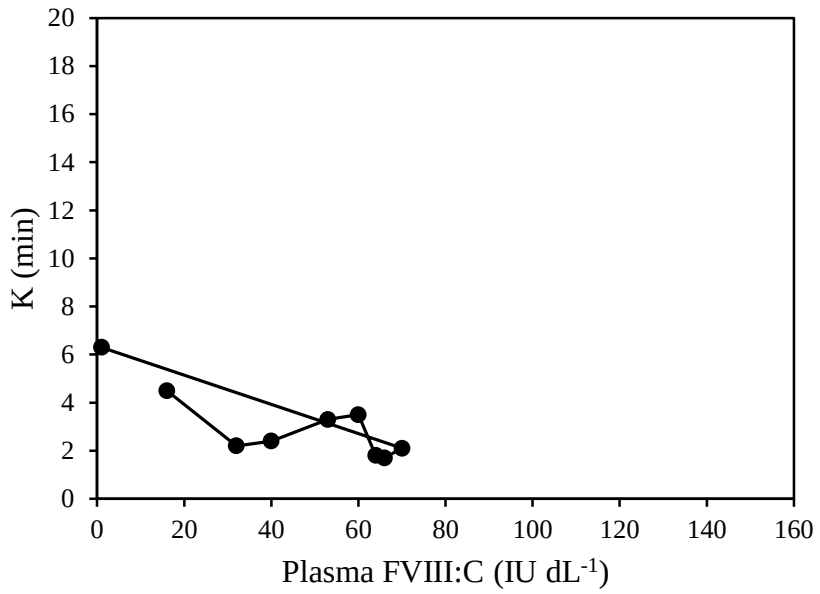
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



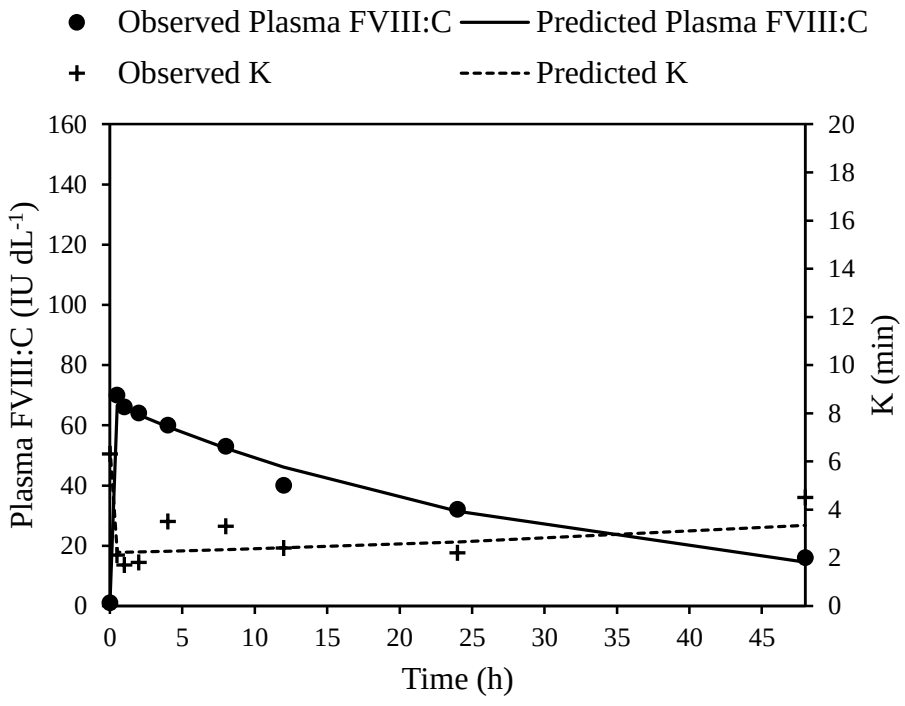
K-Time Profile



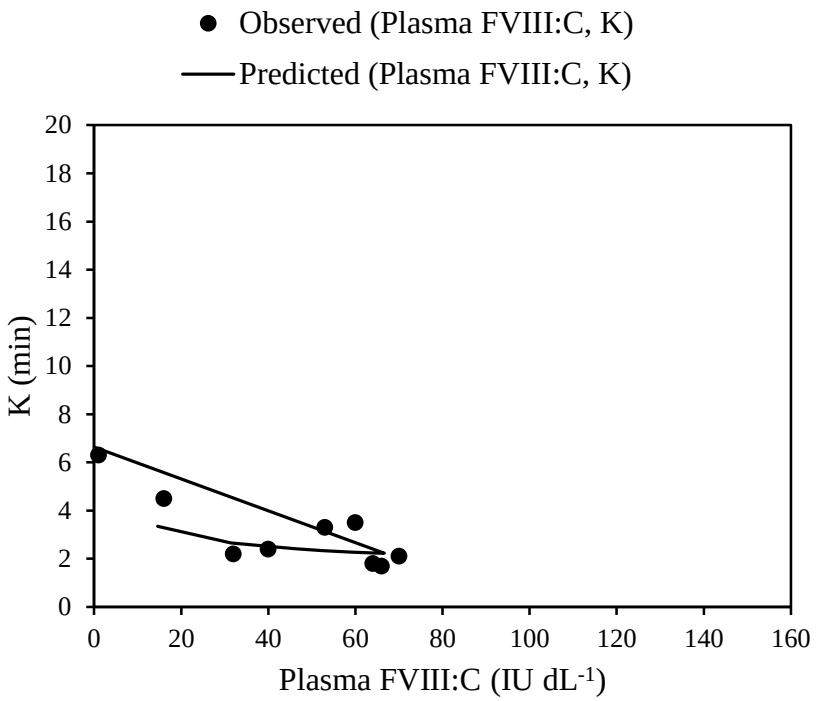
K-Plasma FVIII:C Profile



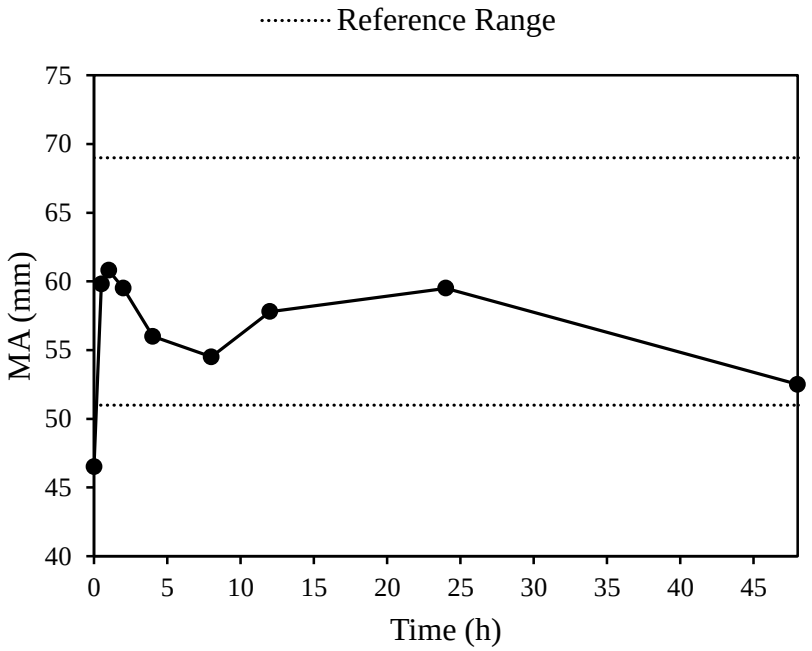
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

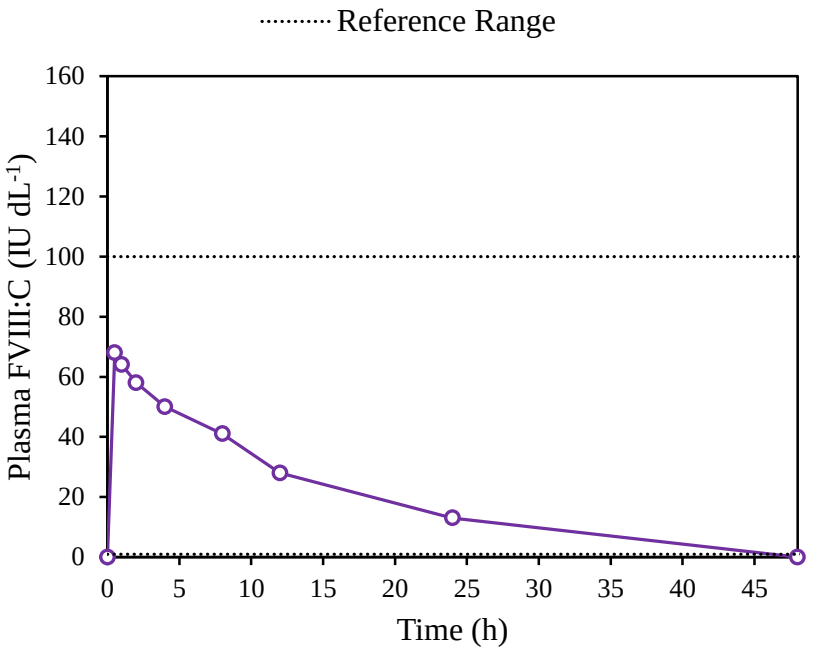


MA-Time Profile

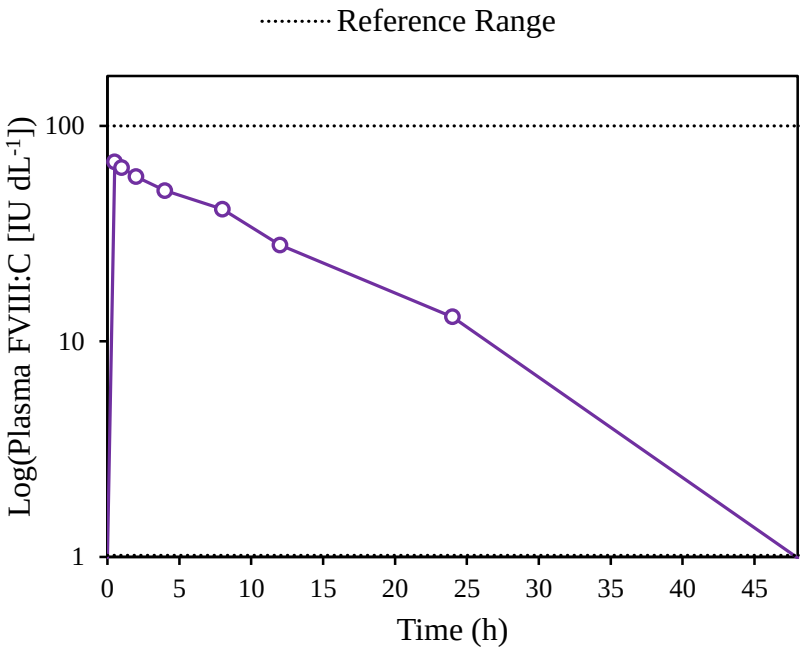


Appendix 6. Individual Profiles for Patient 2

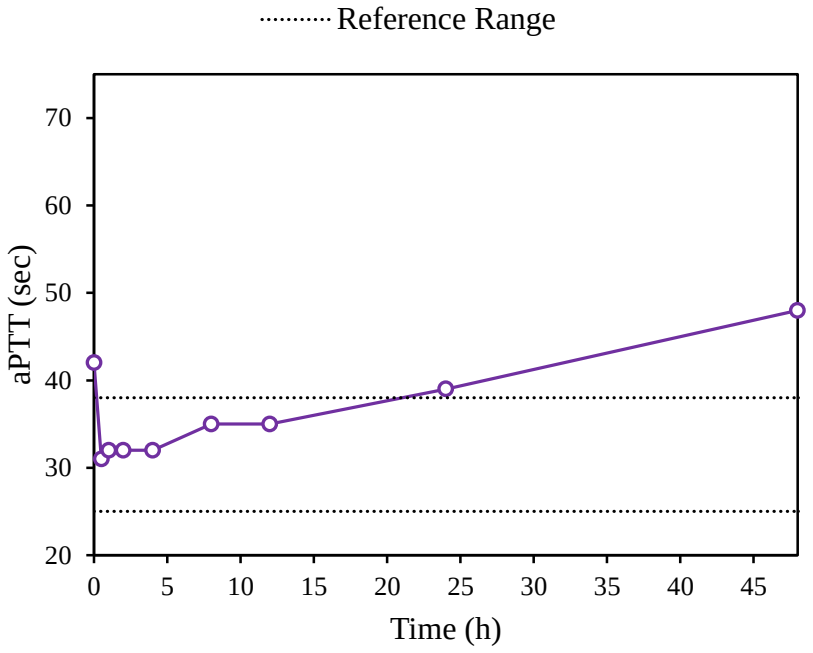
Plasma FVIII:C-Time Profiles



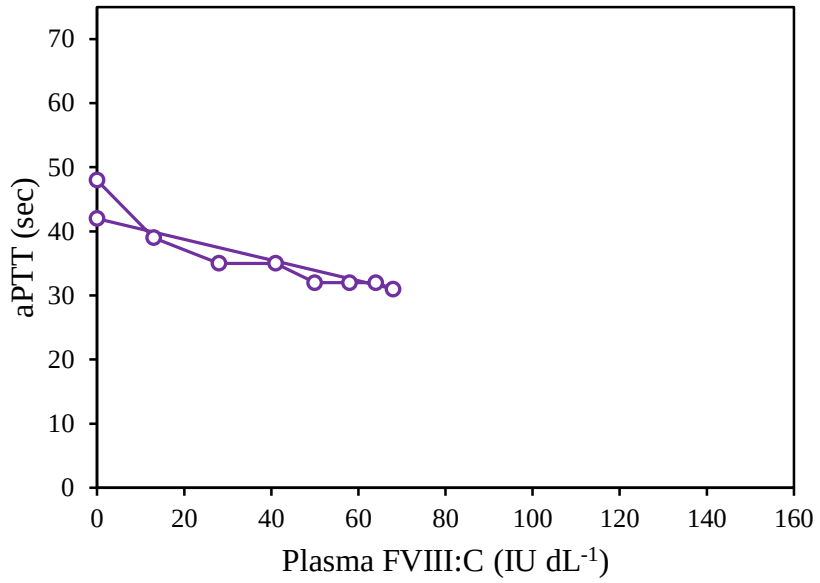
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



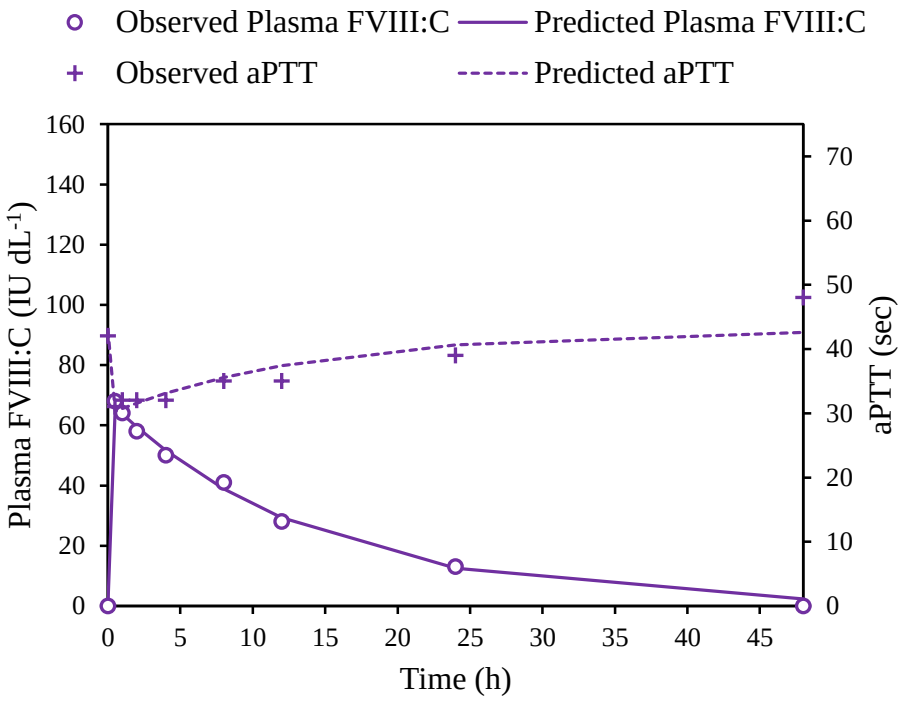
aPTT-Time Profile



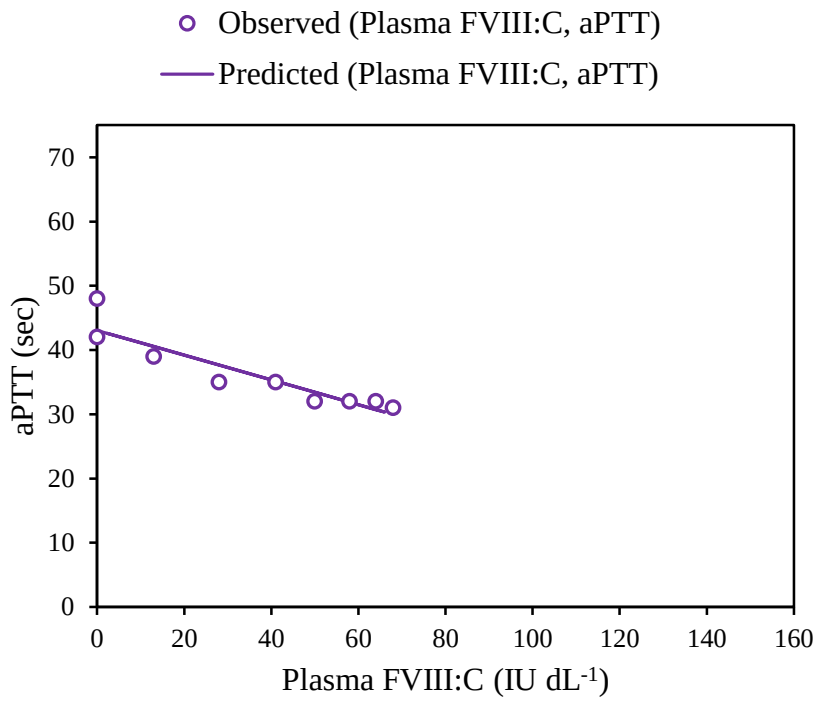
aPTT-Plasma FVIII:C Profile



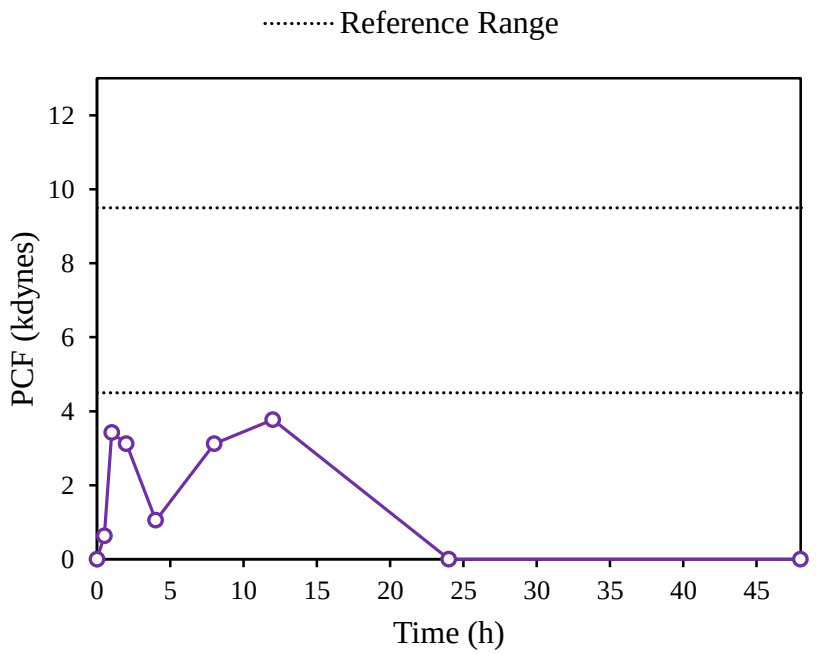
aPTT PK/PD Linear Model Time Dependent Profile



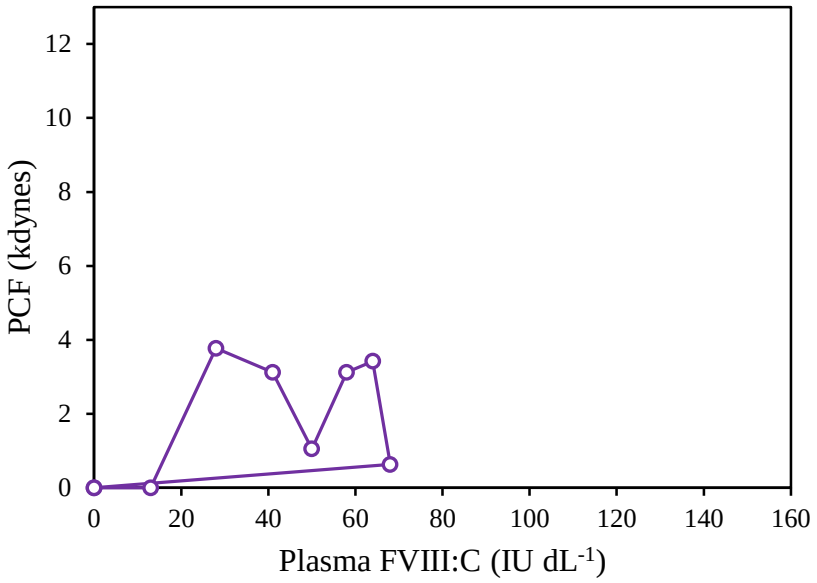
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



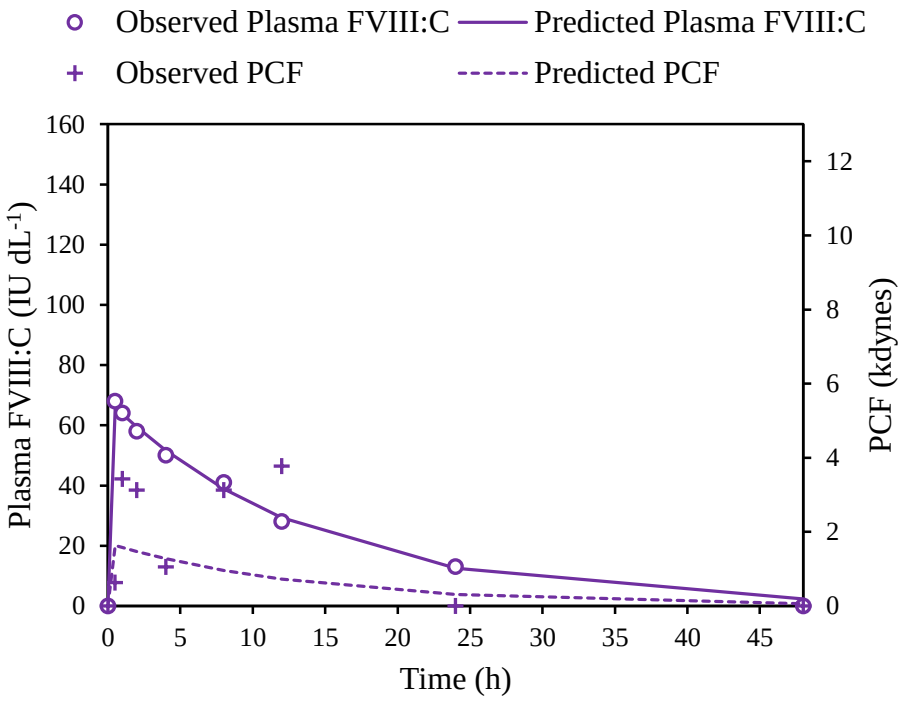
PCF-Time Profile



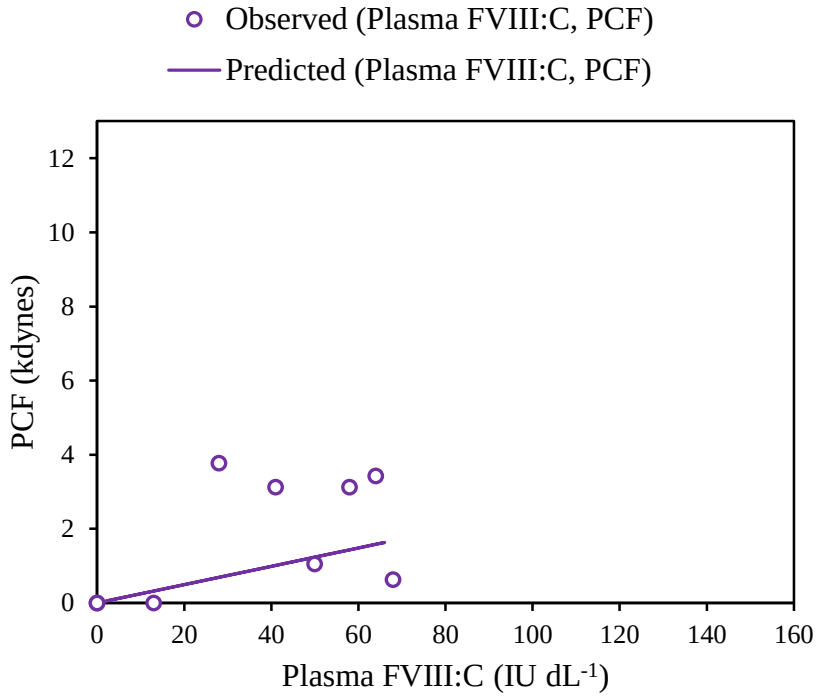
PCF-Plasma FVIII:C Profile



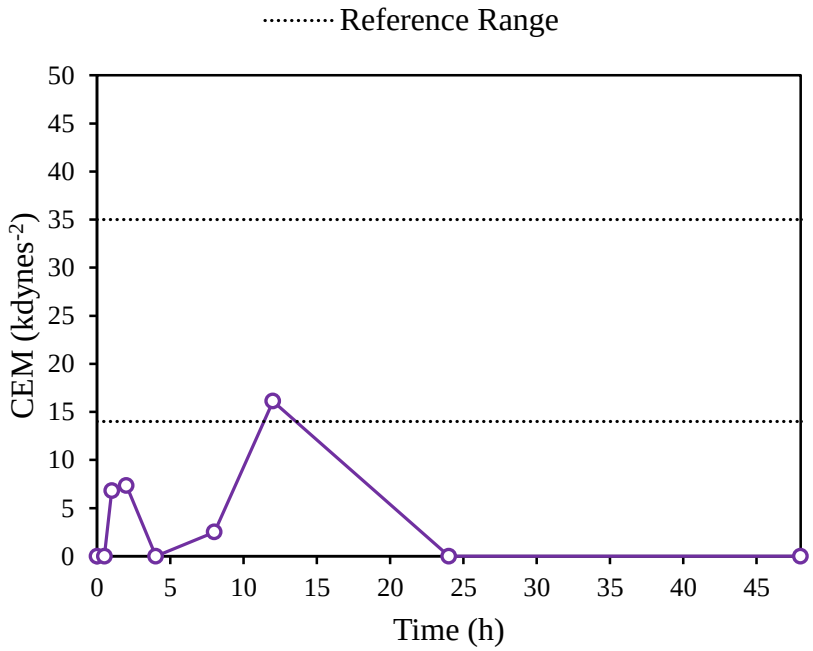
PCF PK/PD Linear Model Time Dependent Profile



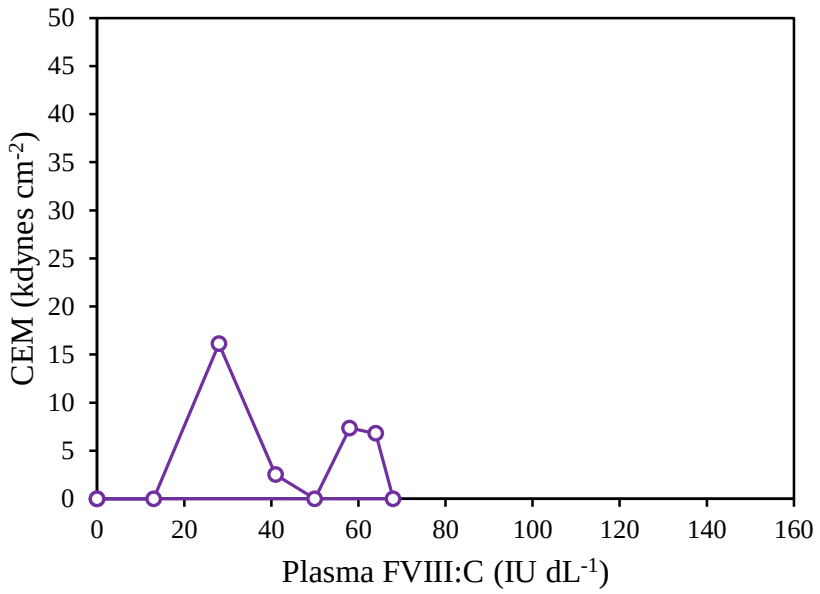
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



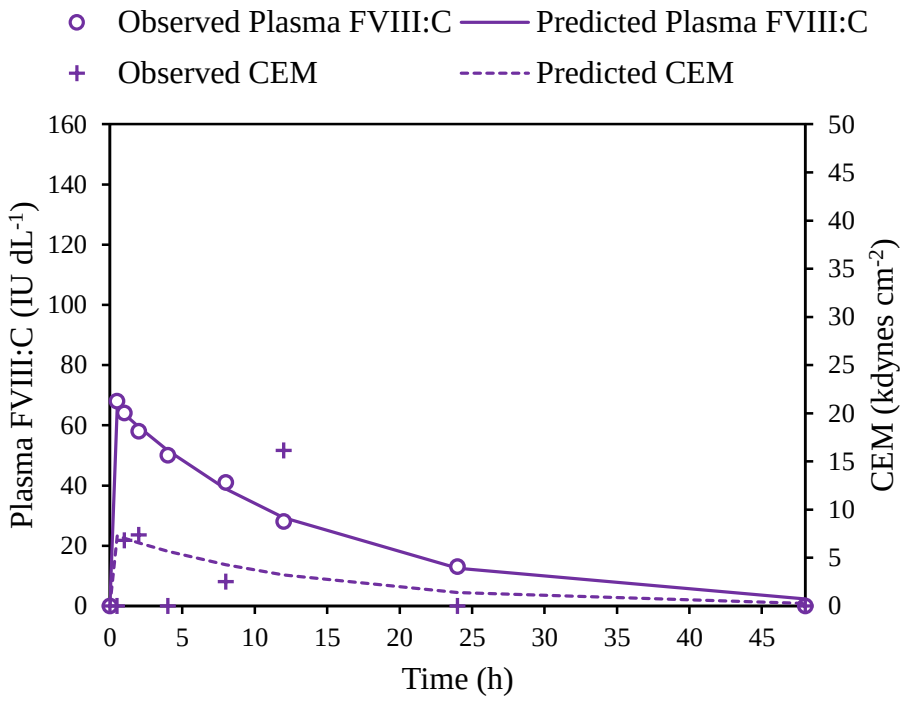
CEM-Time Profile



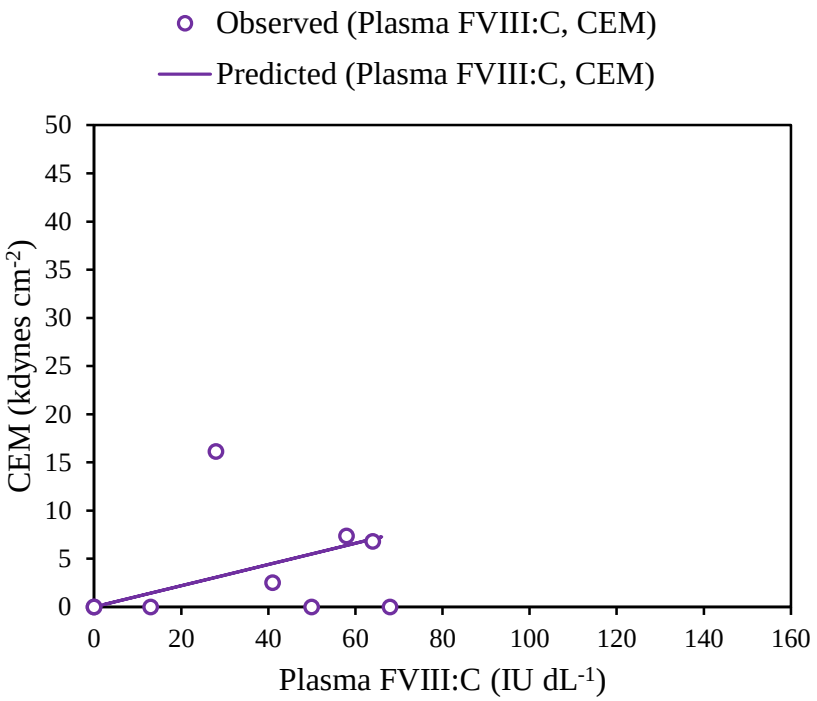
CEM-Plasma FVIII:C Profile



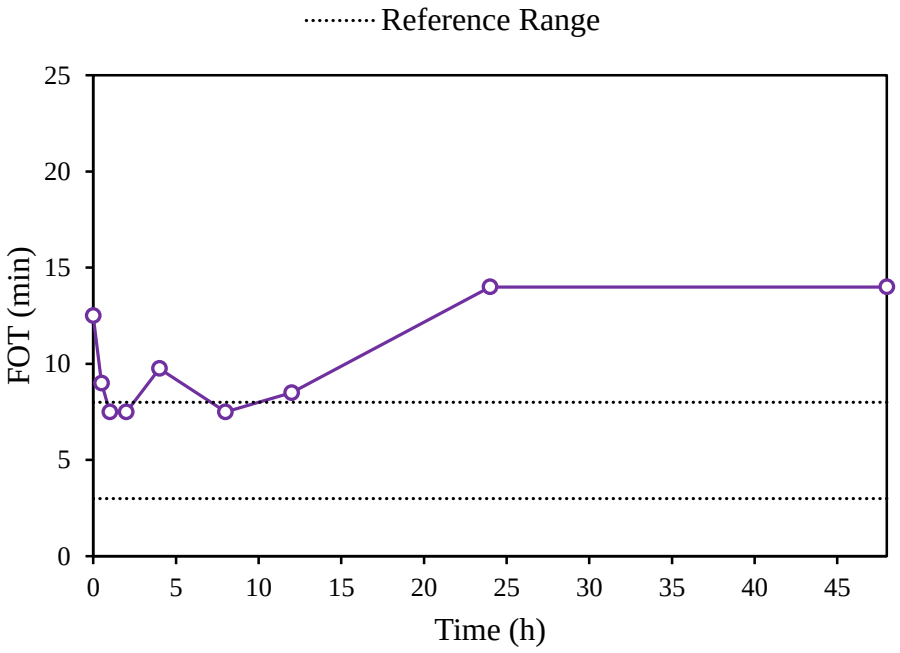
CEM PK/PD Linear Model Time Dependent Profile



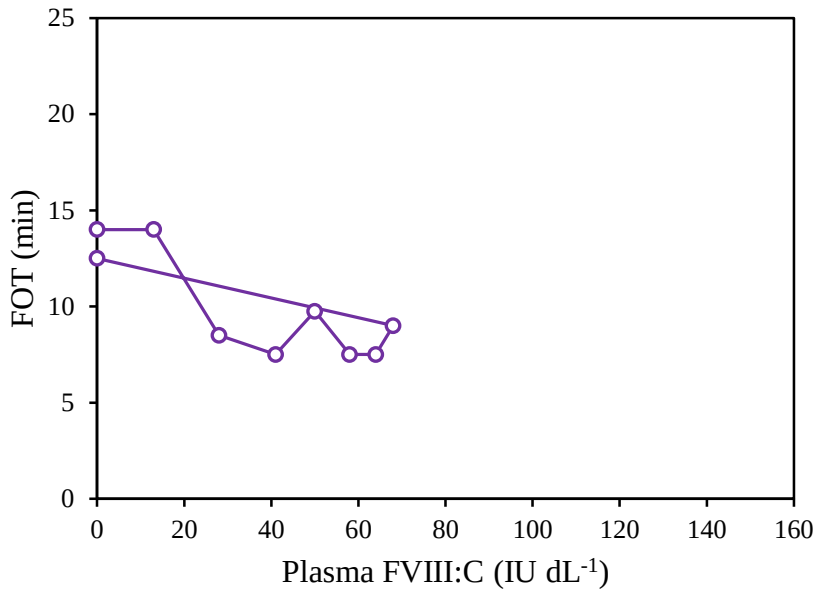
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



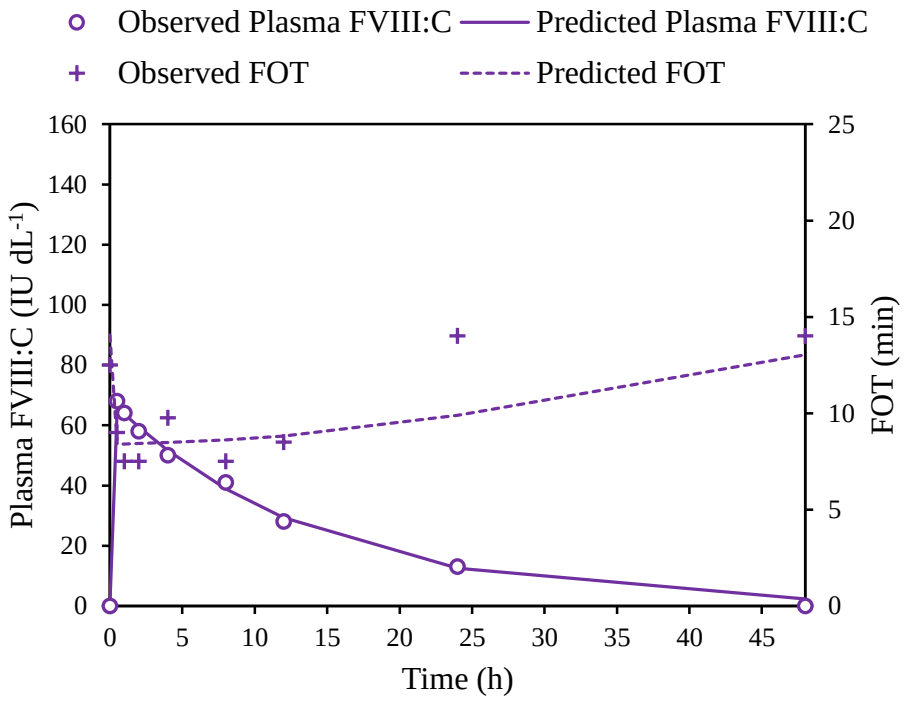
FOT-Time Profile



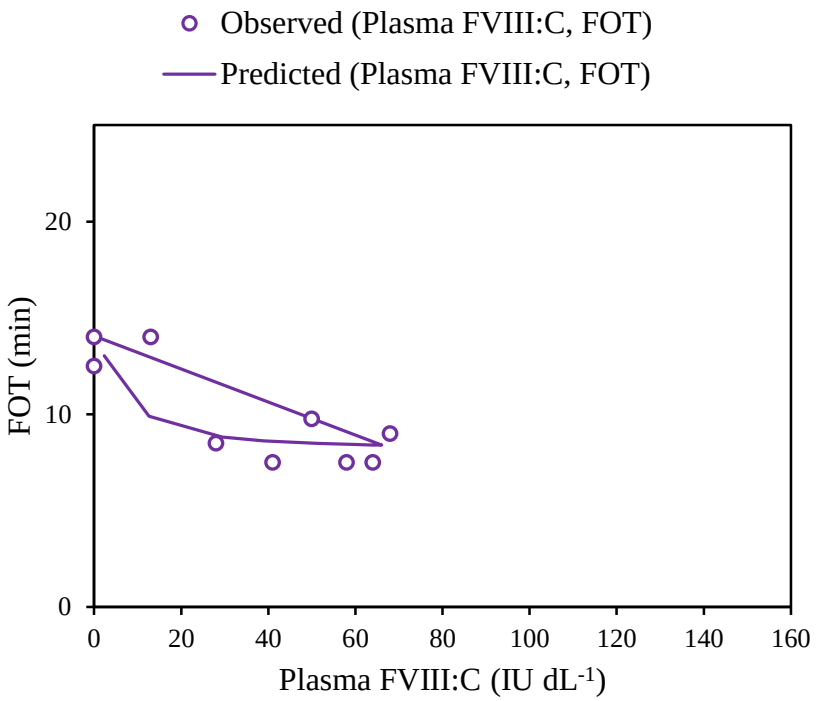
FOT-Plasma FVIII:C Profile



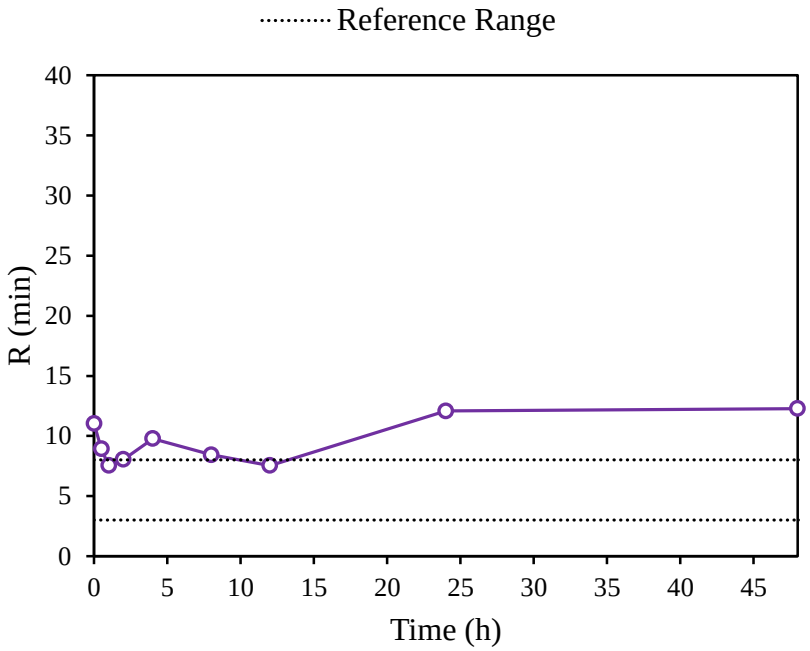
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



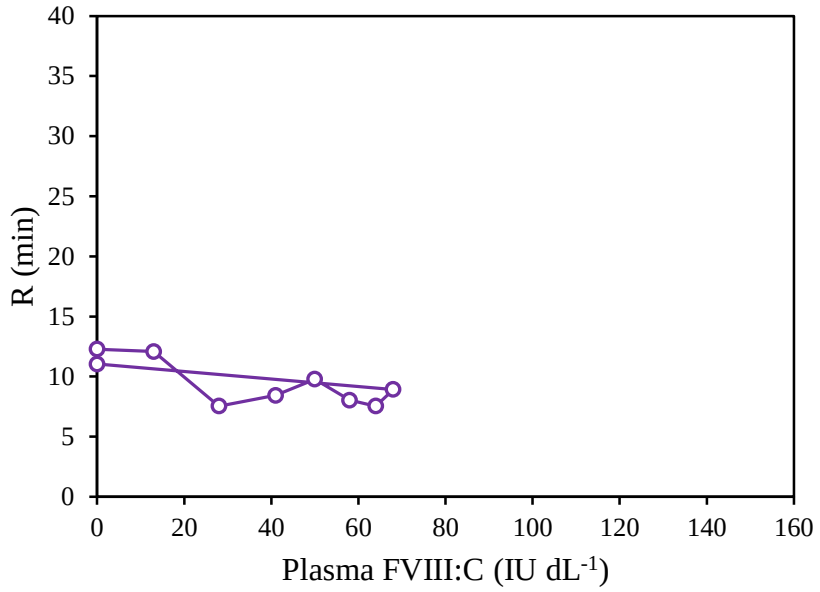
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



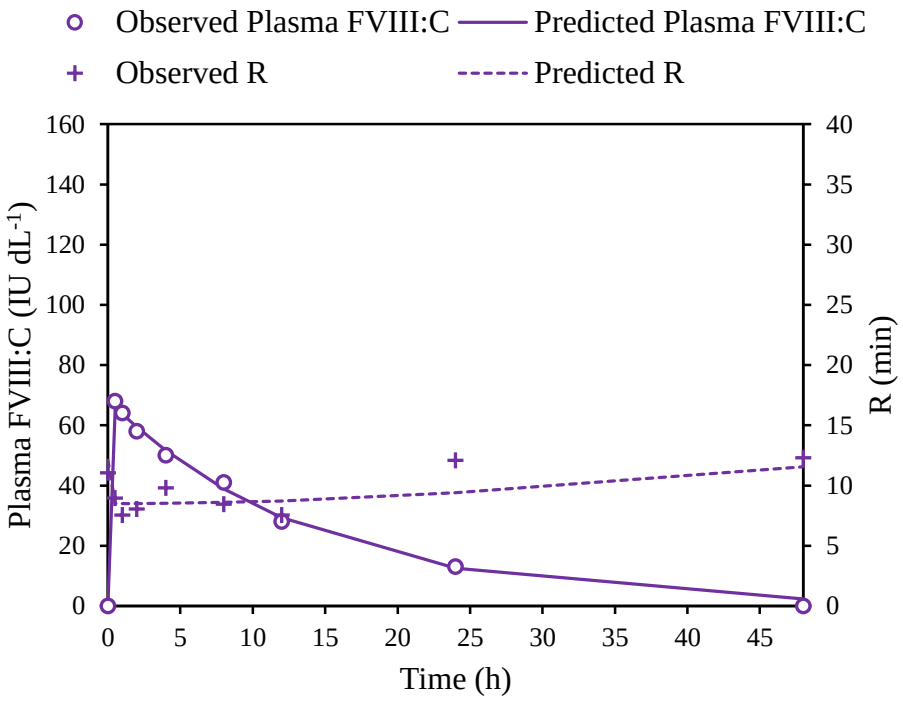
R-Time Profile



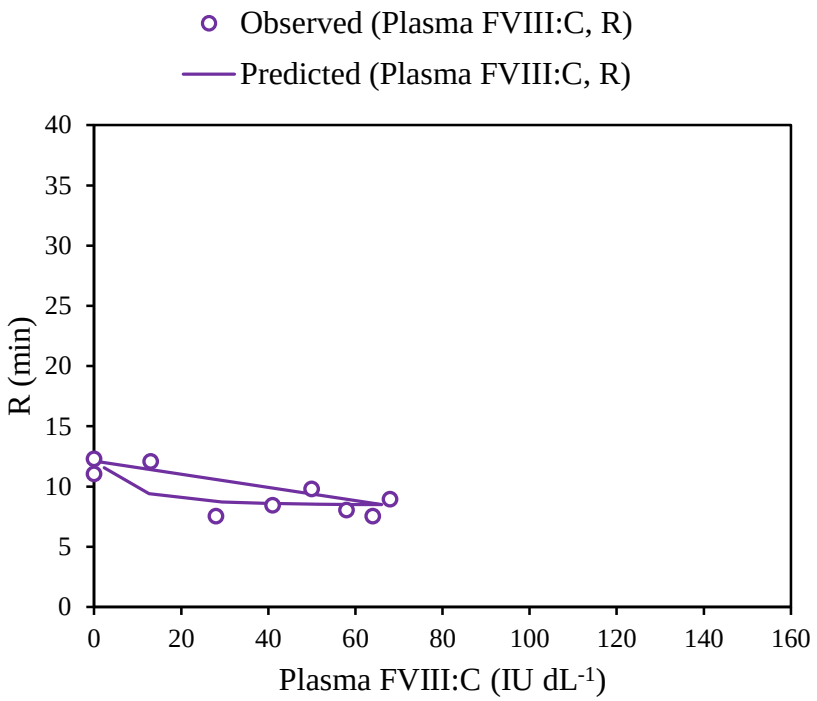
R-Plasma FVIII:C Profile



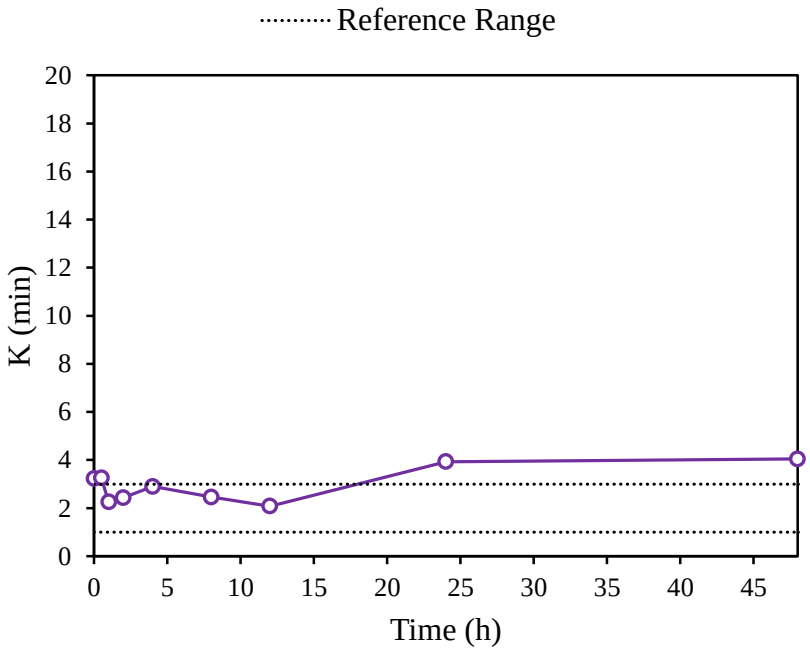
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



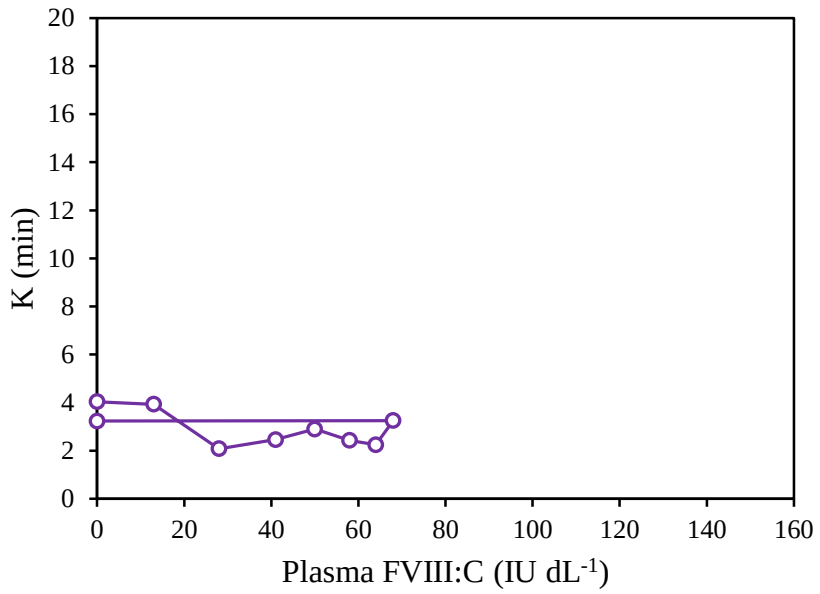
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



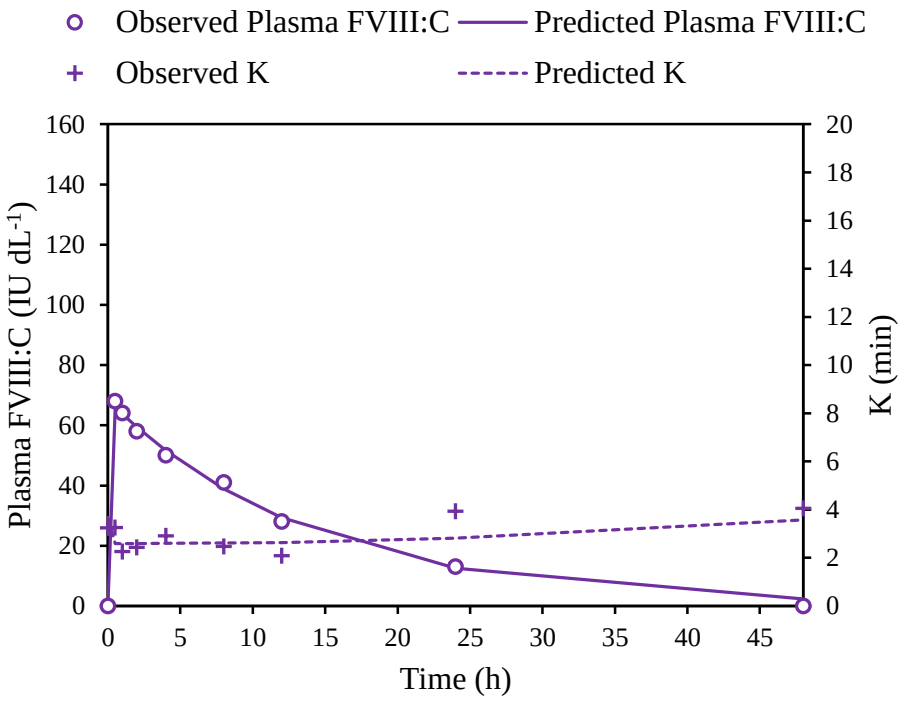
K-Time Profile



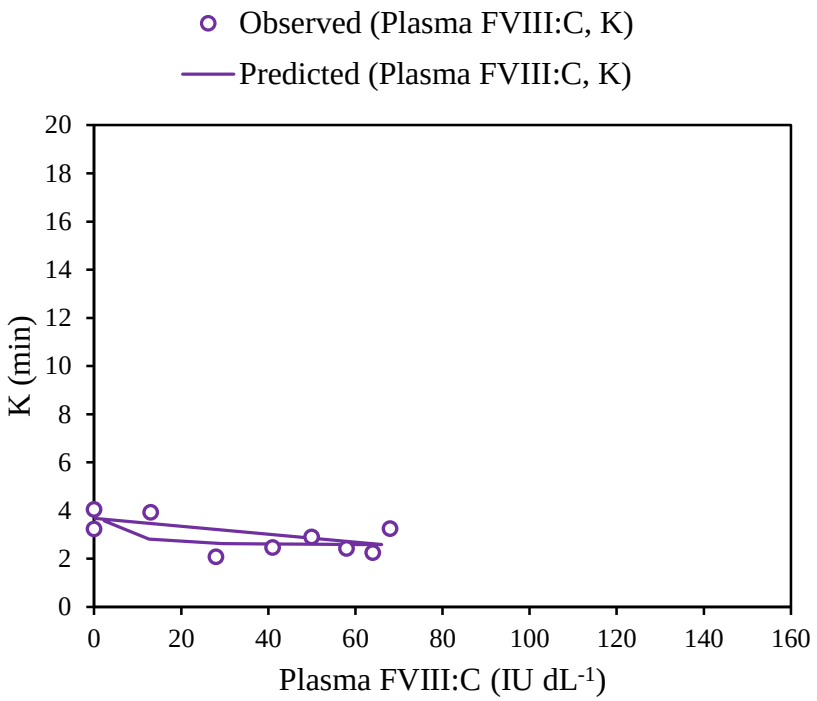
K-Plasma FVIII:C Profile



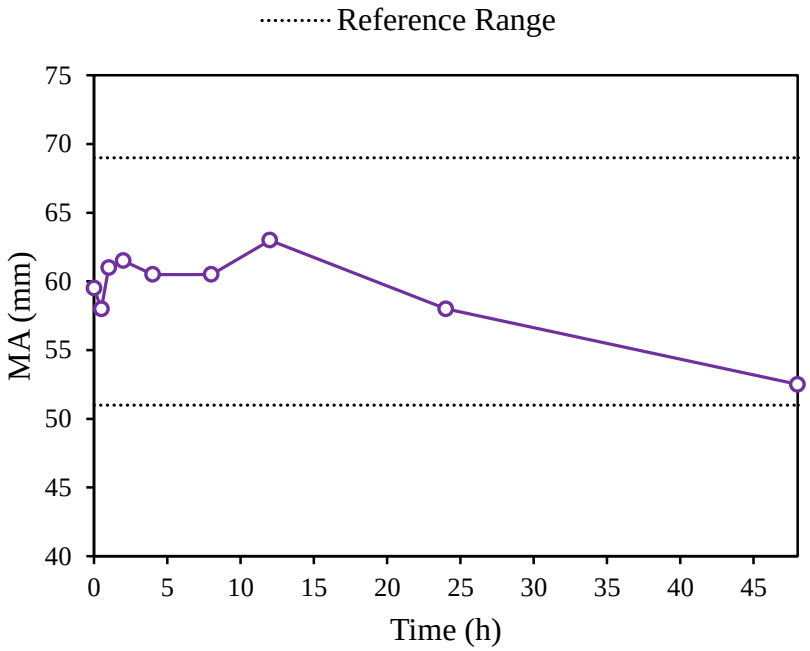
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

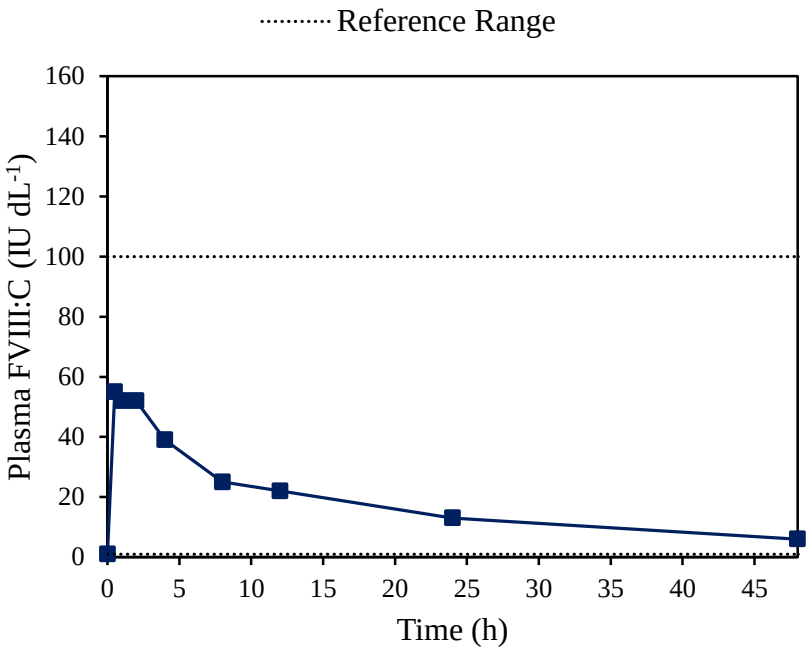


MA-Time Profile

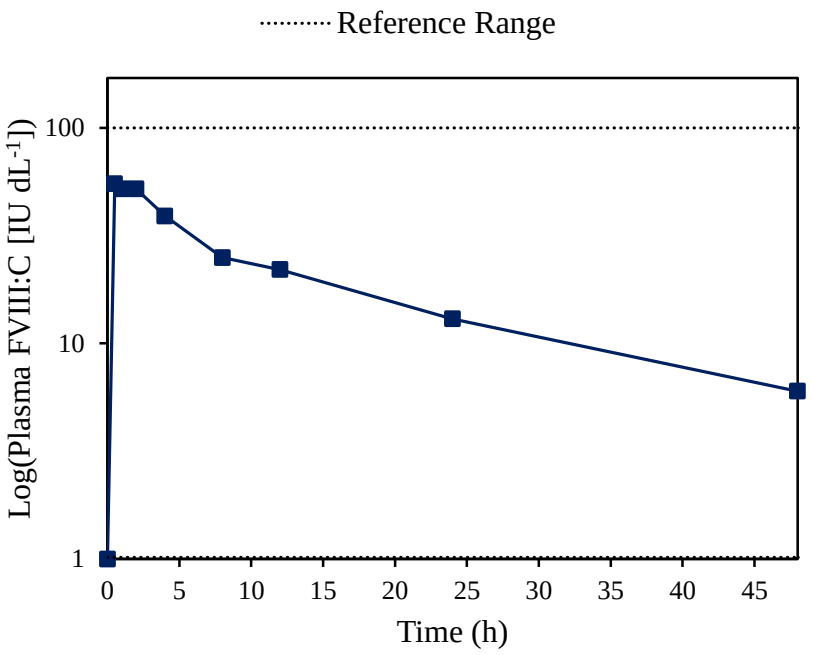


Appendix 7. Individual Profiles for Patient 3

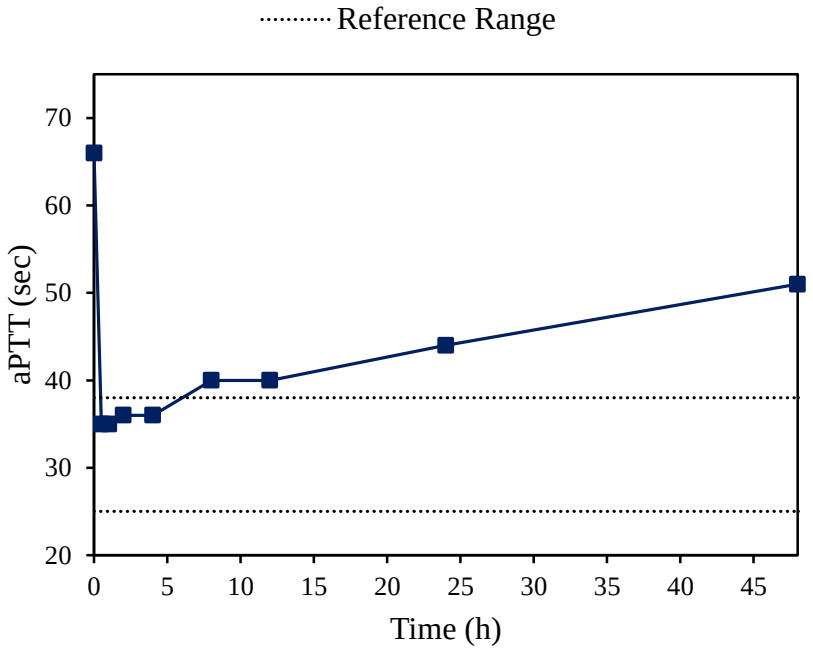
Plasma FVIII:C-Time Profiles



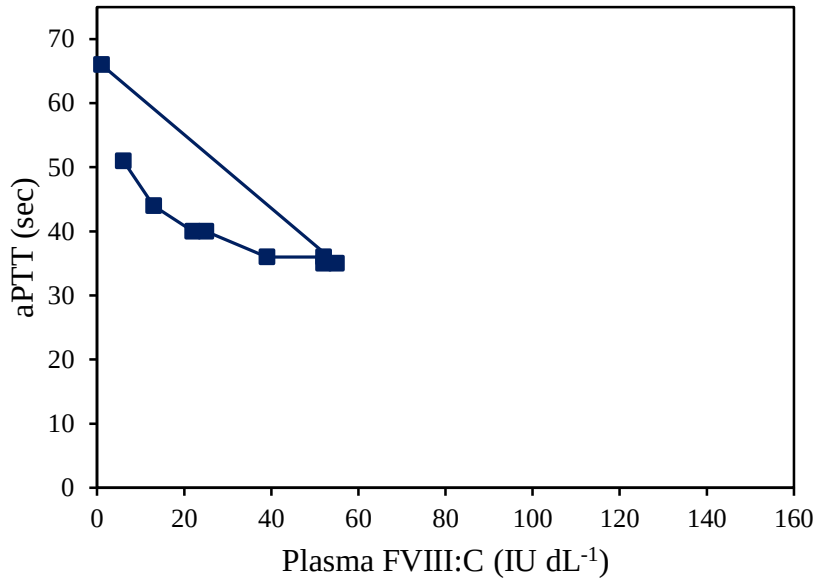
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



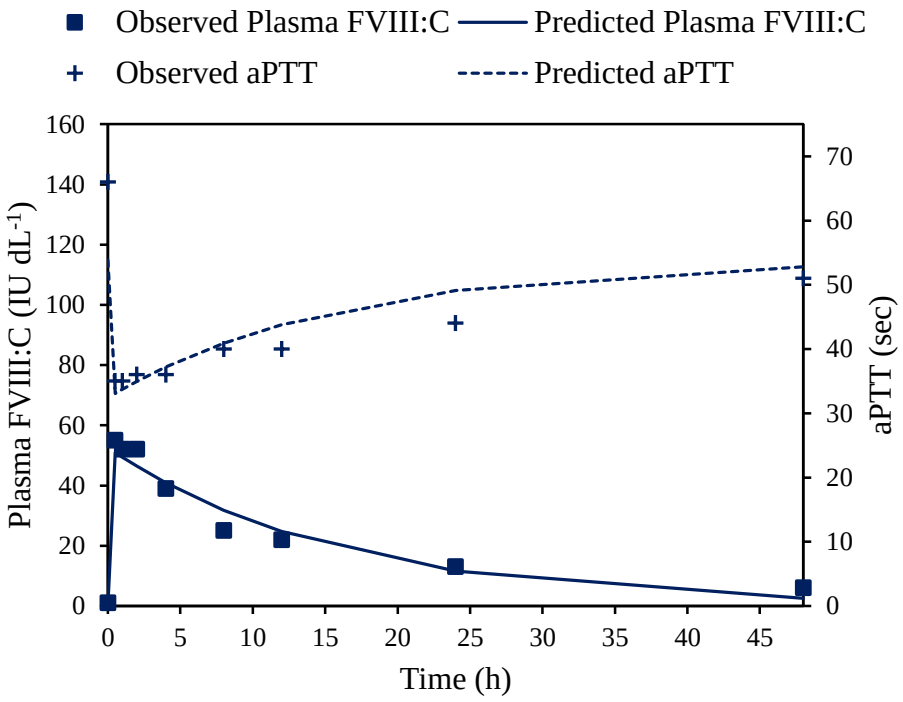
aPTT-Time Profile



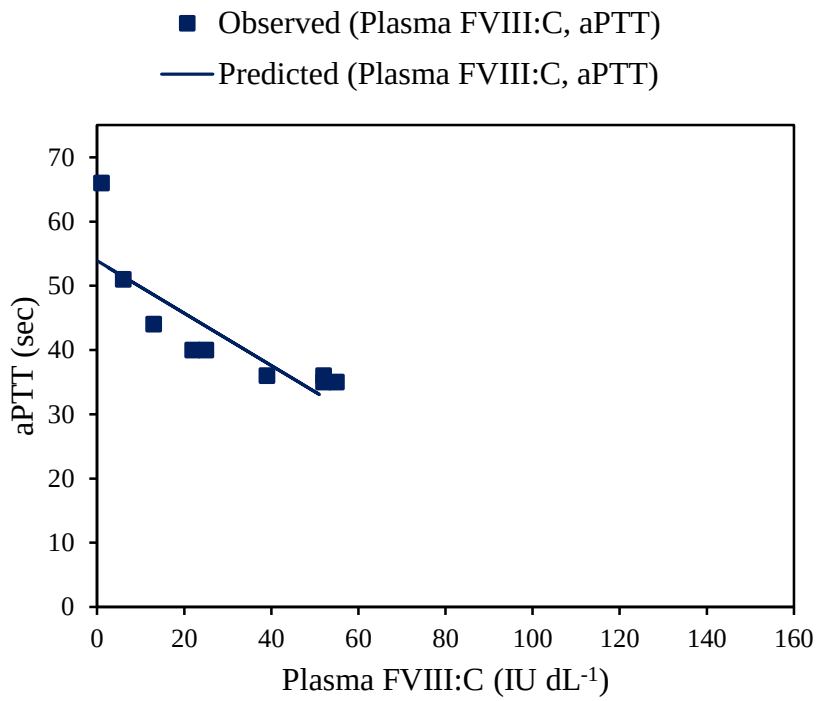
aPTT-Plasma FVIII:C Profile



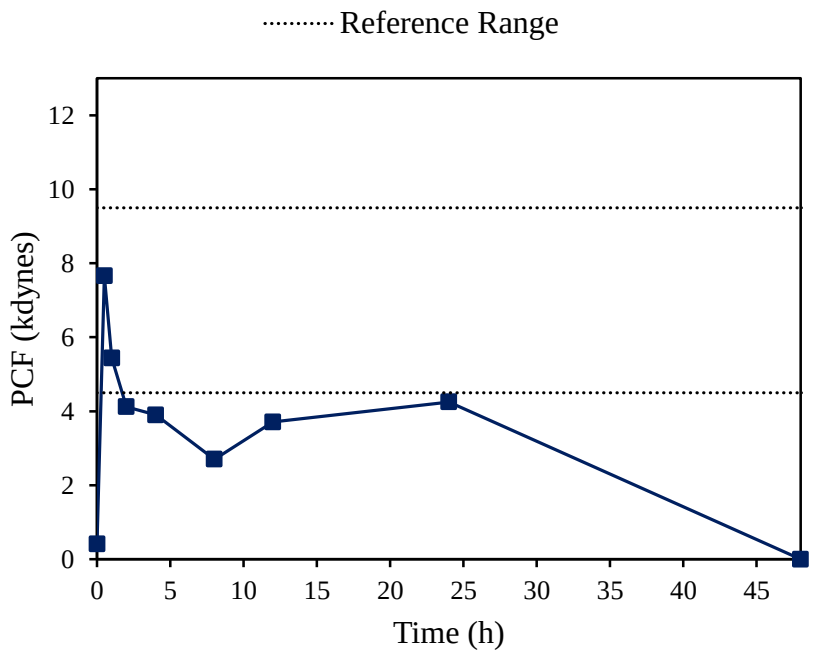
aPTT PK/PD Linear Model Time Dependent Profile



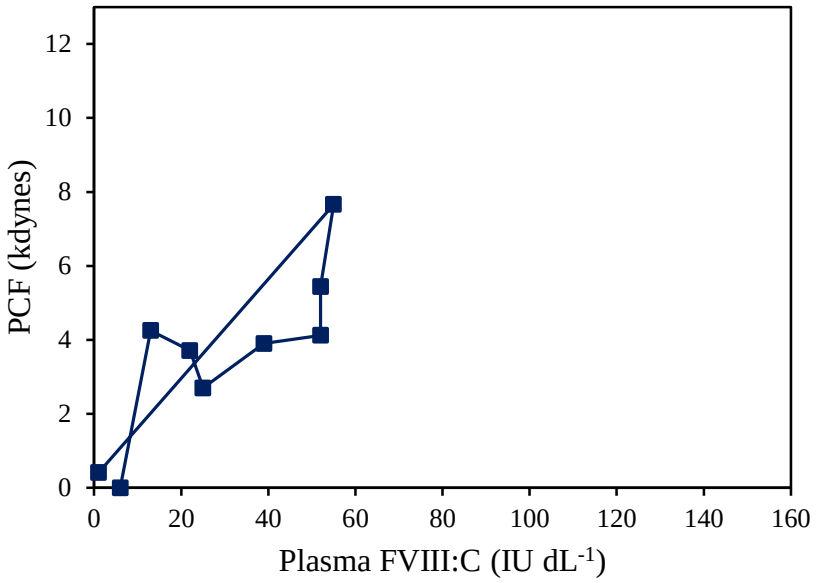
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



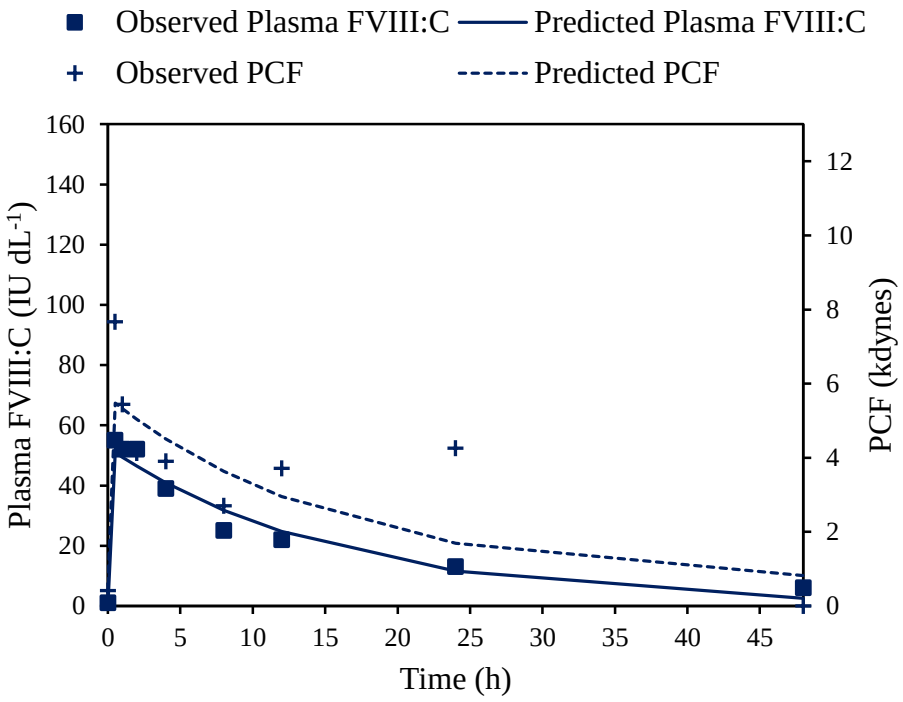
PCF-Time Profile



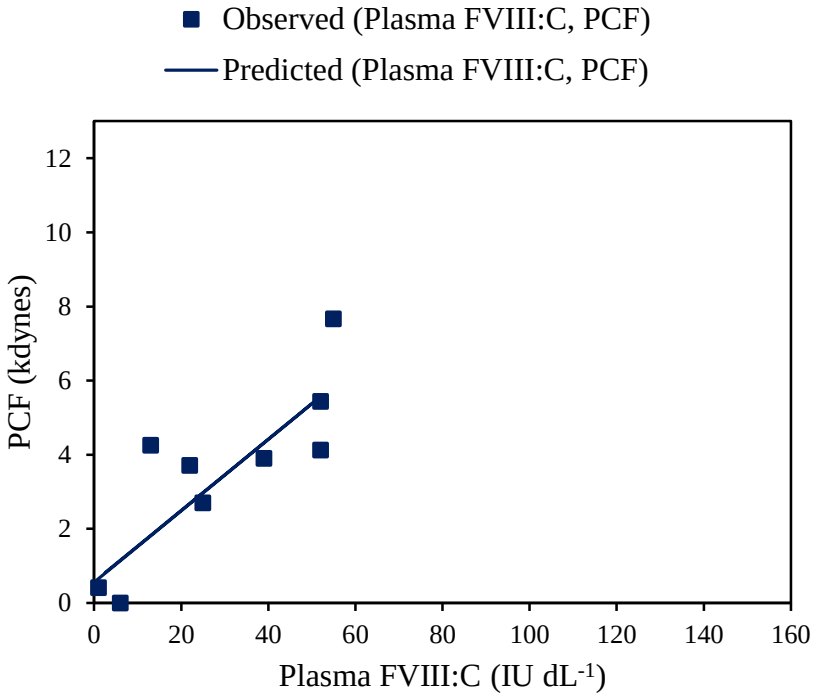
PCF-Plasma FVIII:C Profile



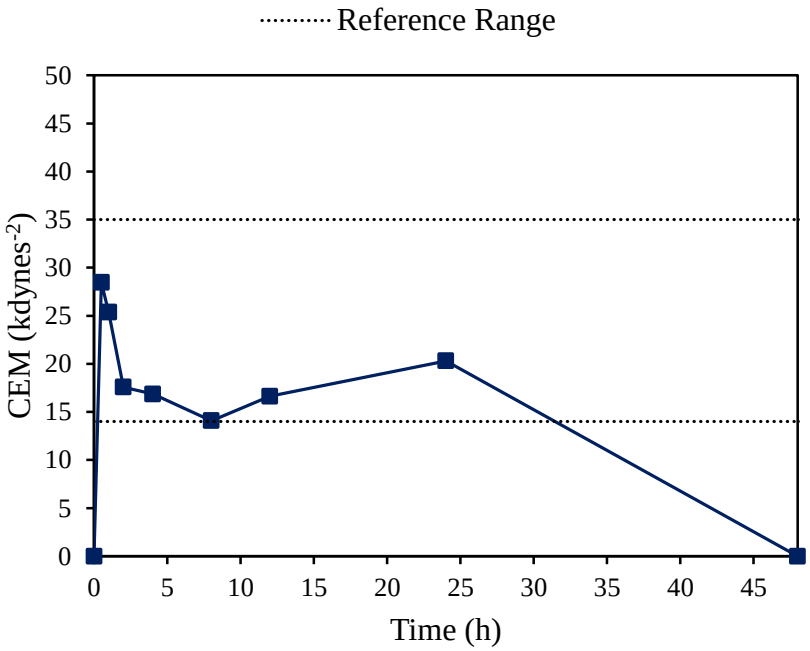
PCF PK/PD Linear Model Time Dependent Profile



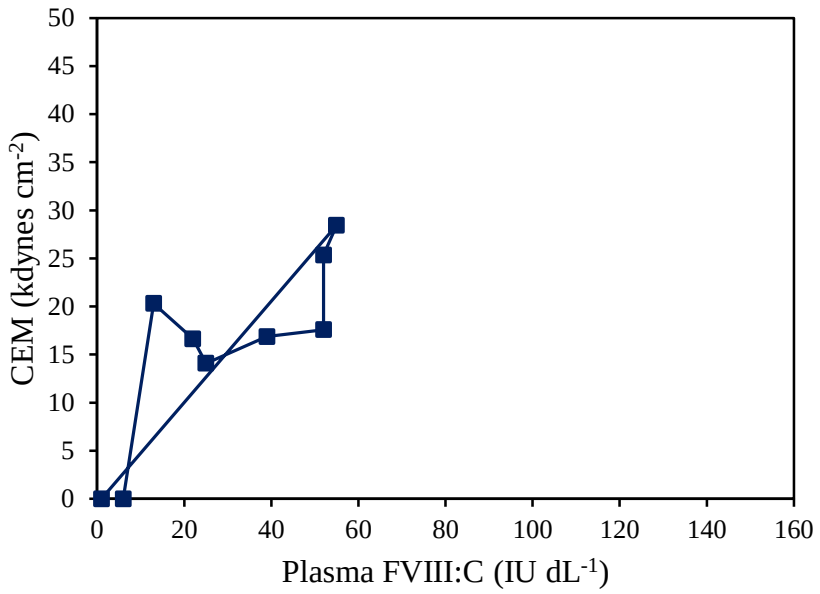
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



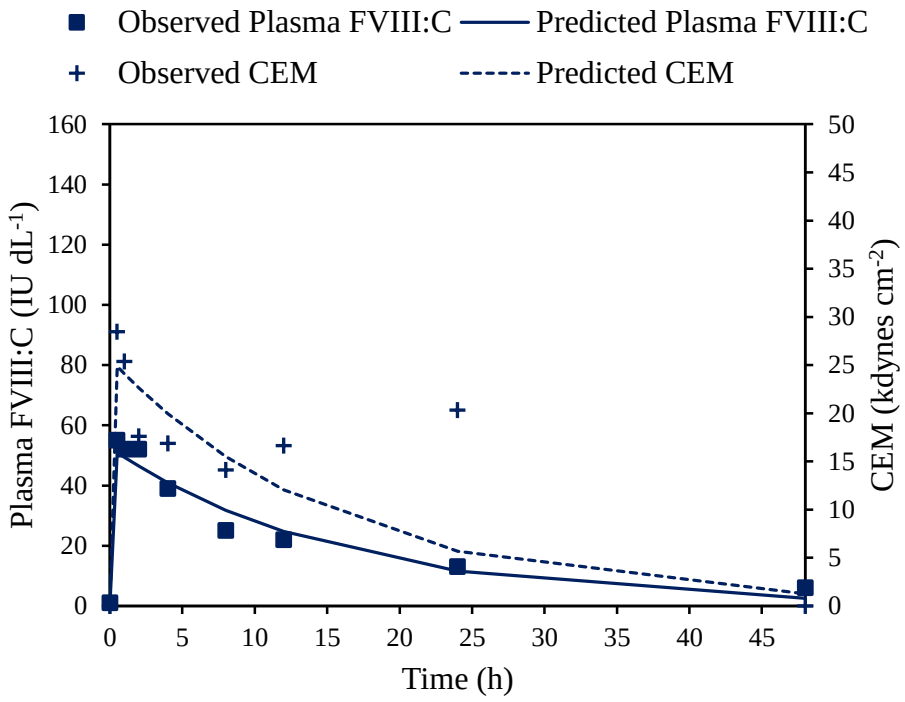
CEM-Time Profile



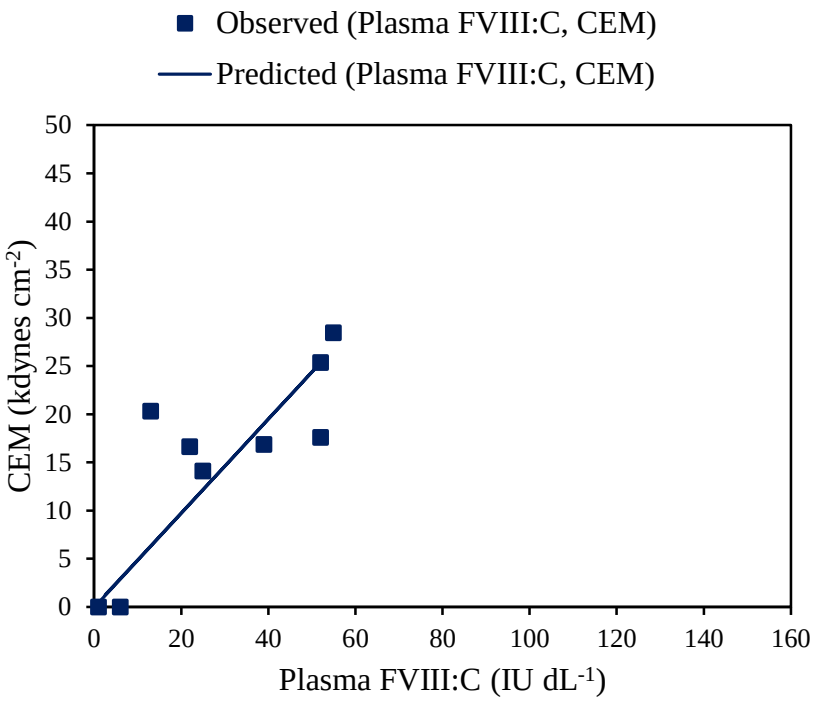
CEM-Plasma FVIII:C Profile



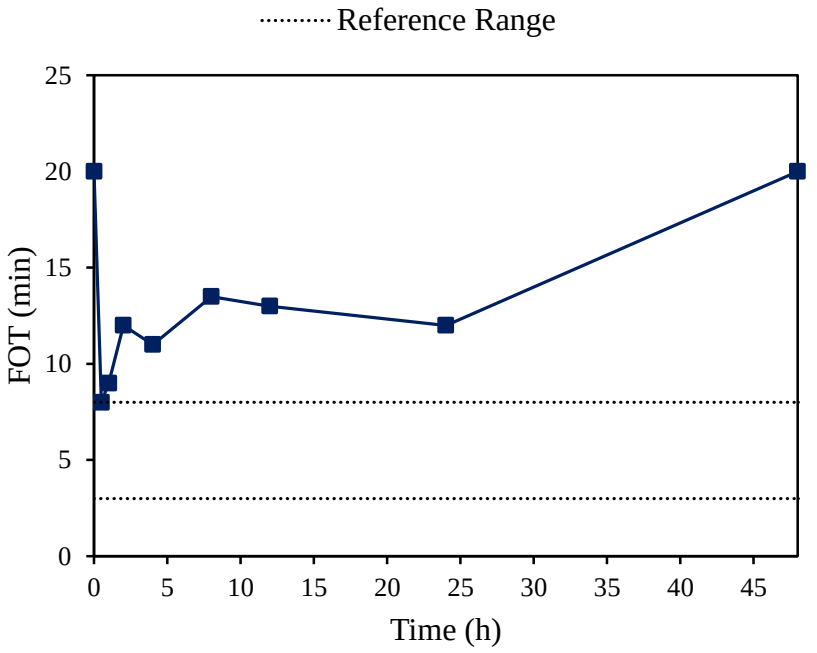
CEM PK/PD Linear Model Time Dependent Profile



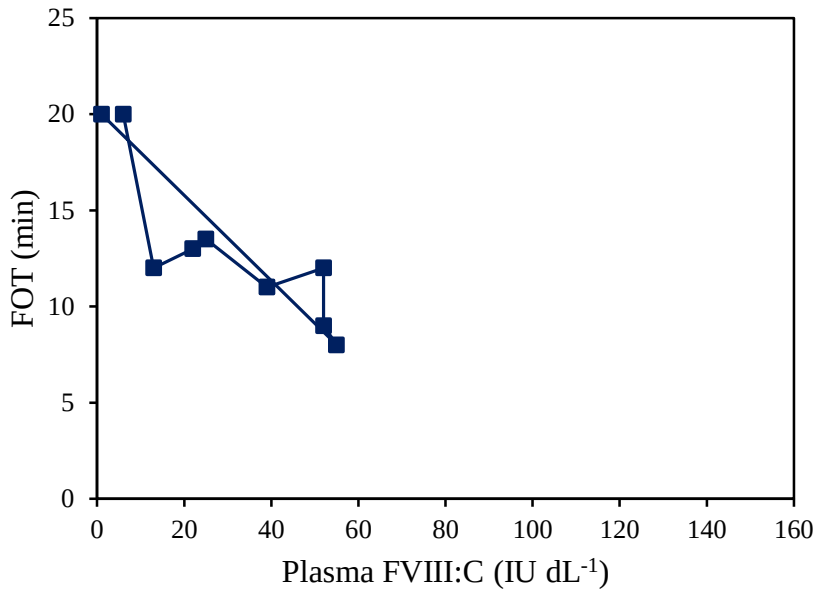
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



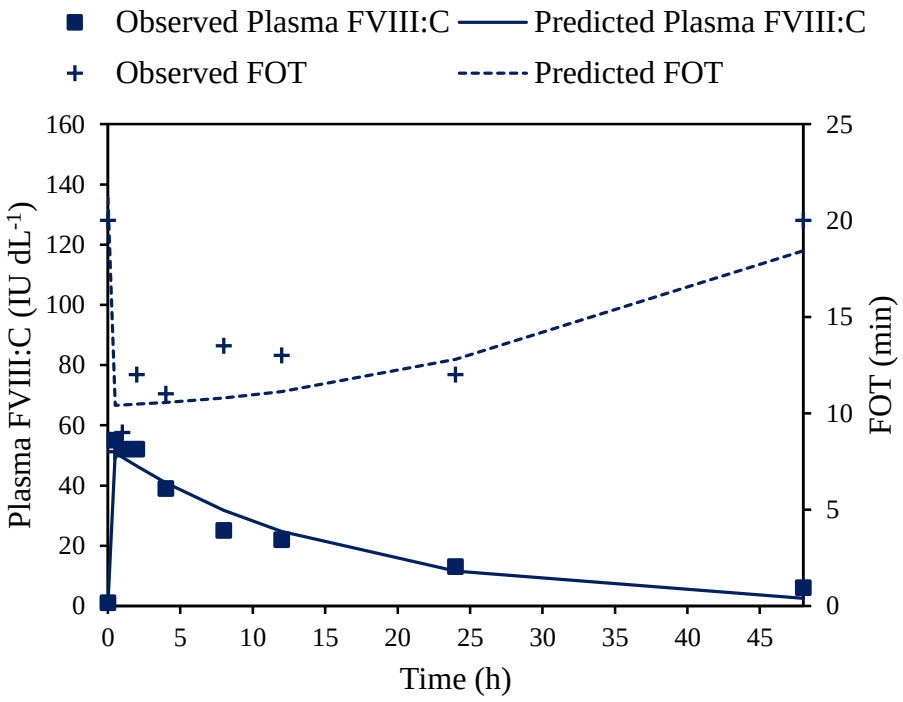
FOT-Time Profile



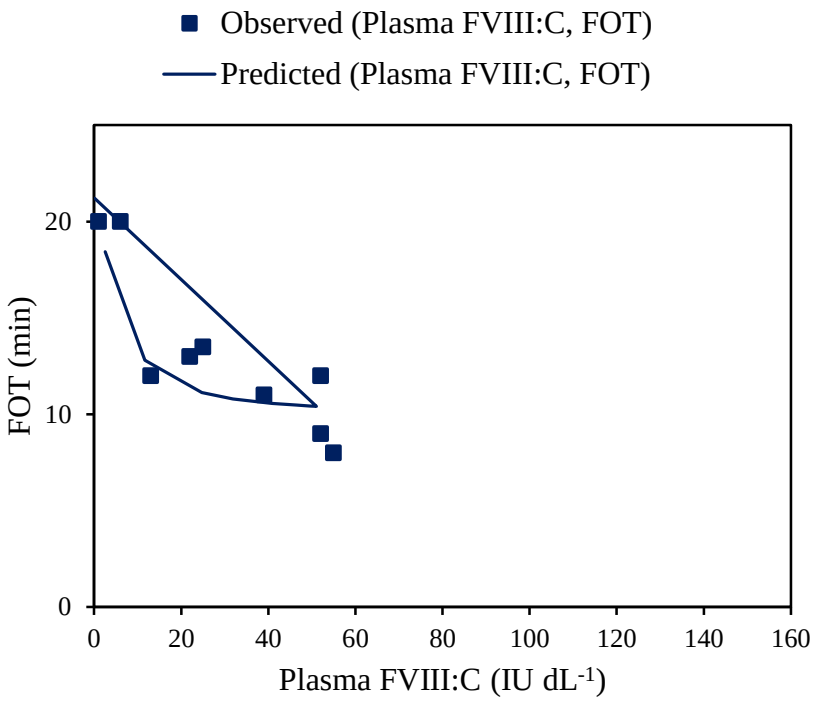
FOT-Plasma FVIII:C Profile



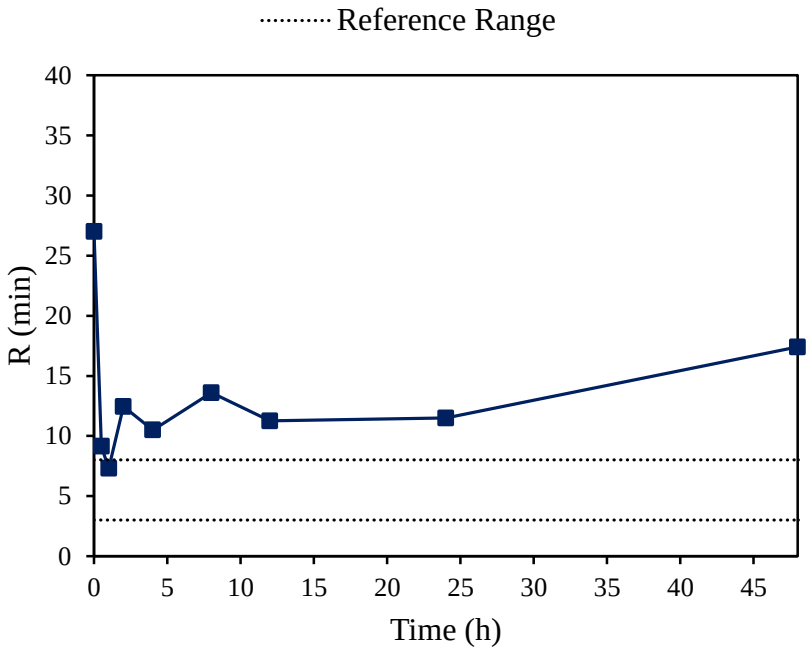
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



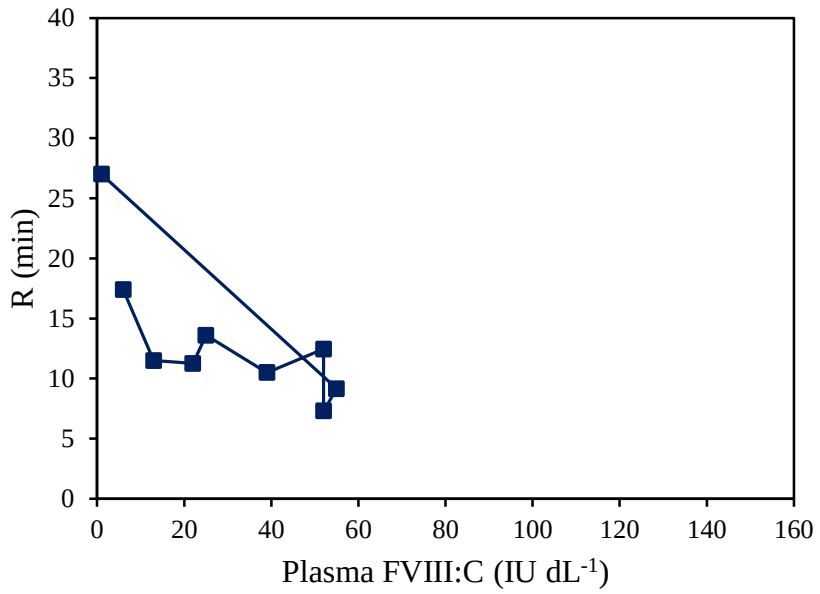
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



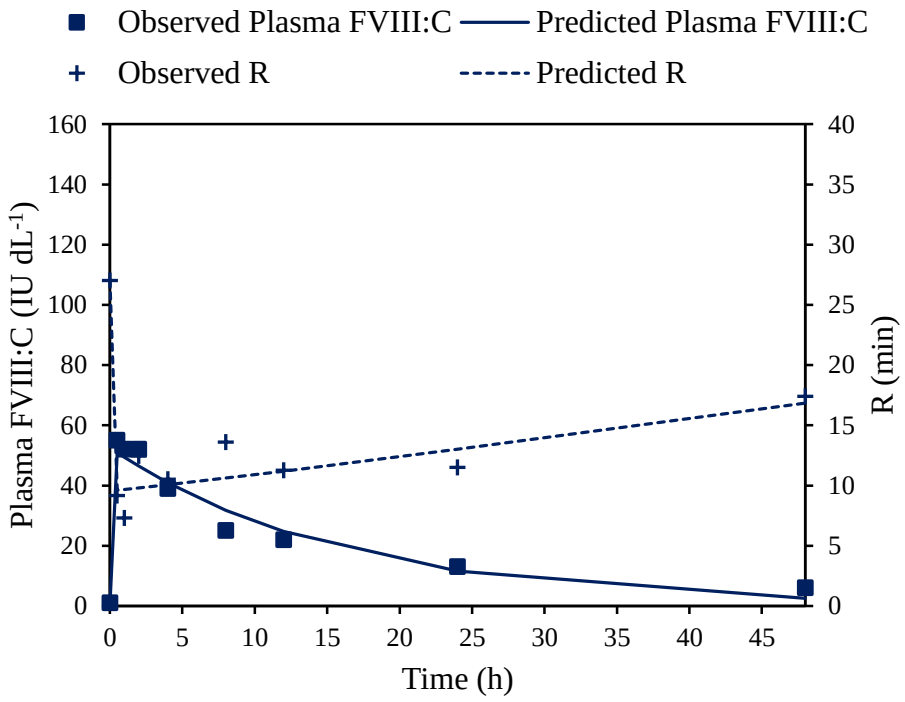
R-Time Profile



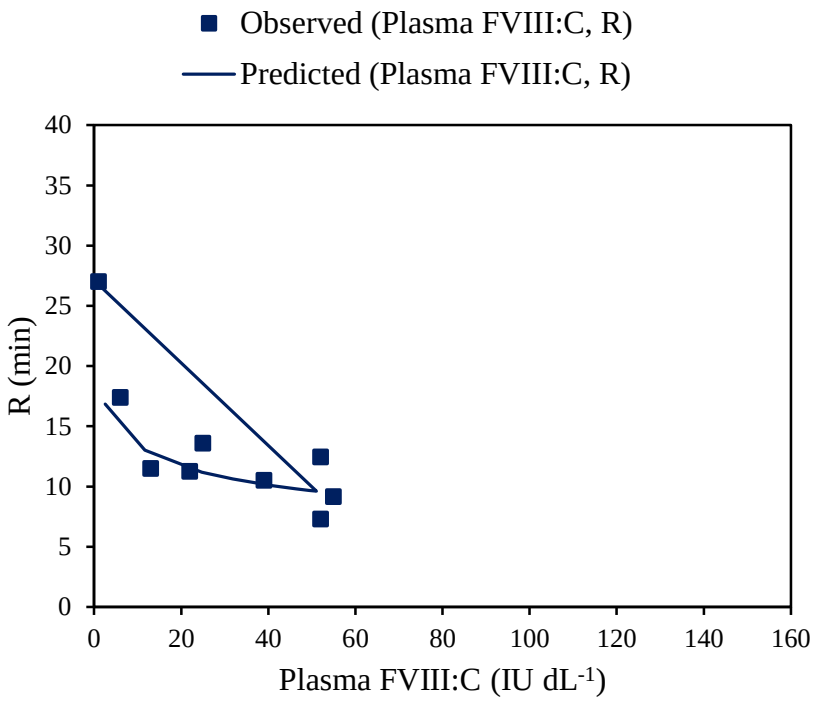
R-Plasma FVIII:C Profile



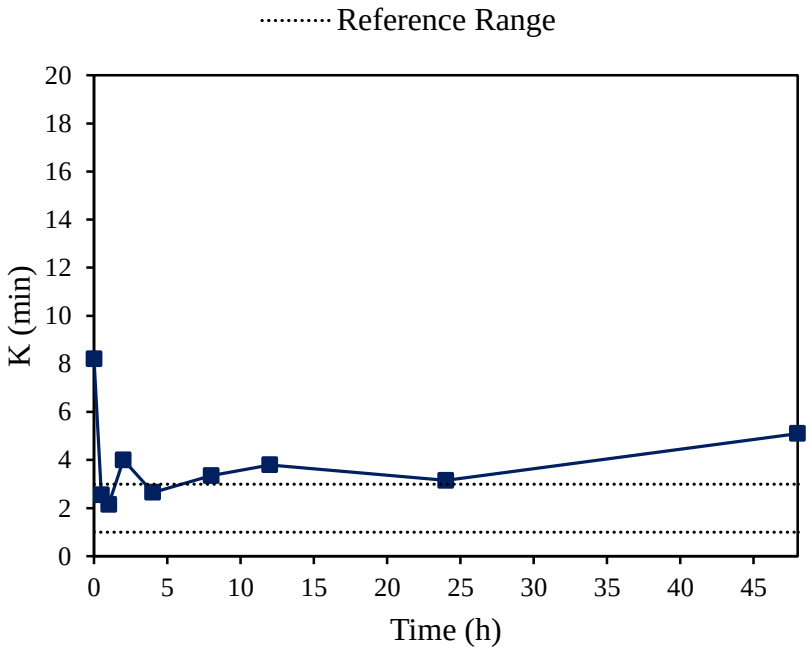
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



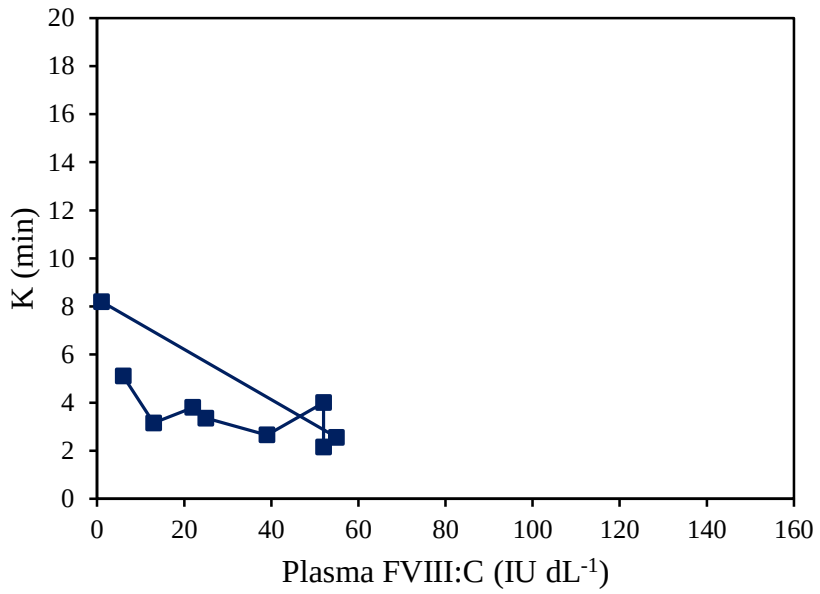
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



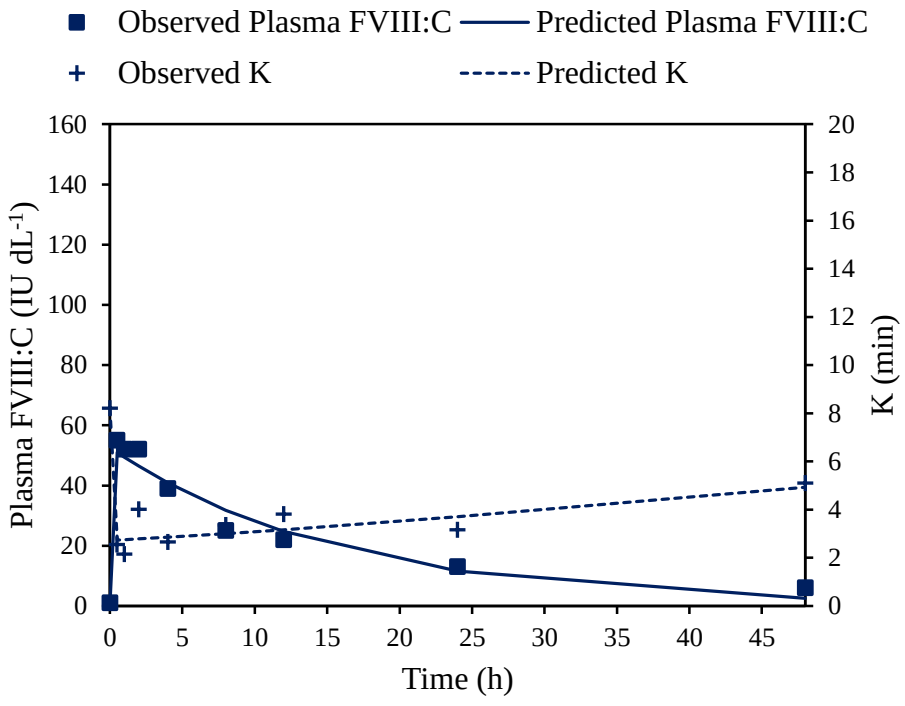
K-Time Profile



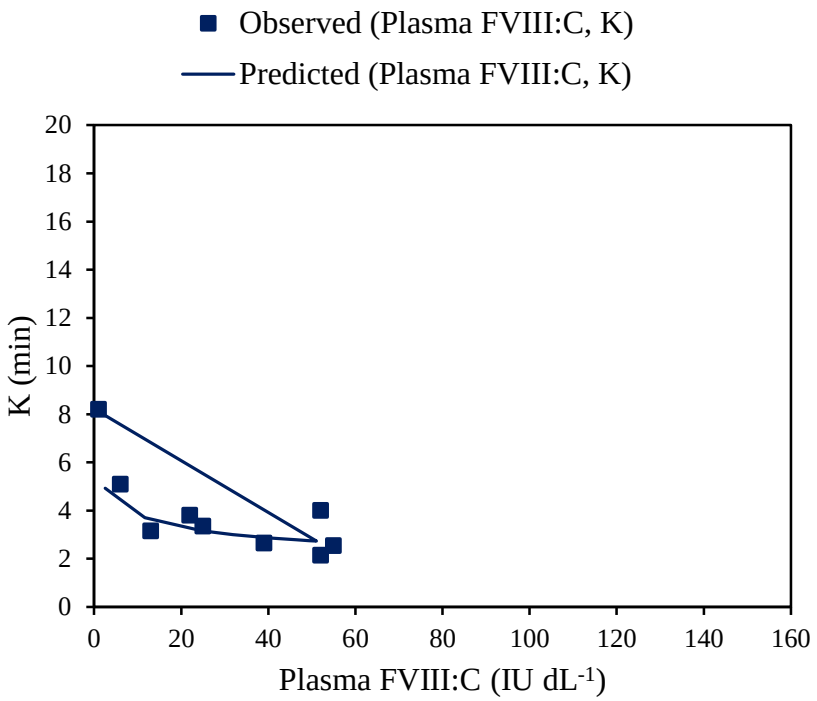
K-Plasma FVIII:C Profile



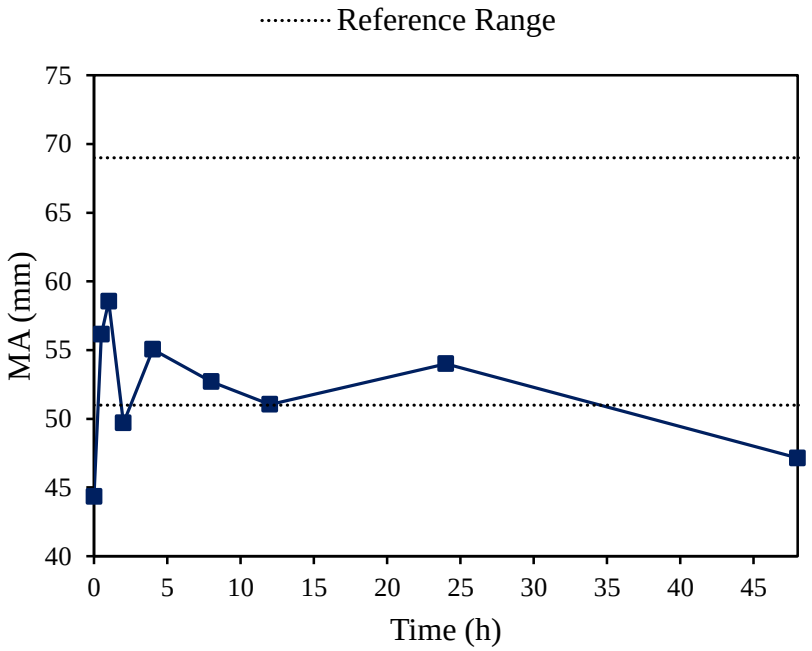
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

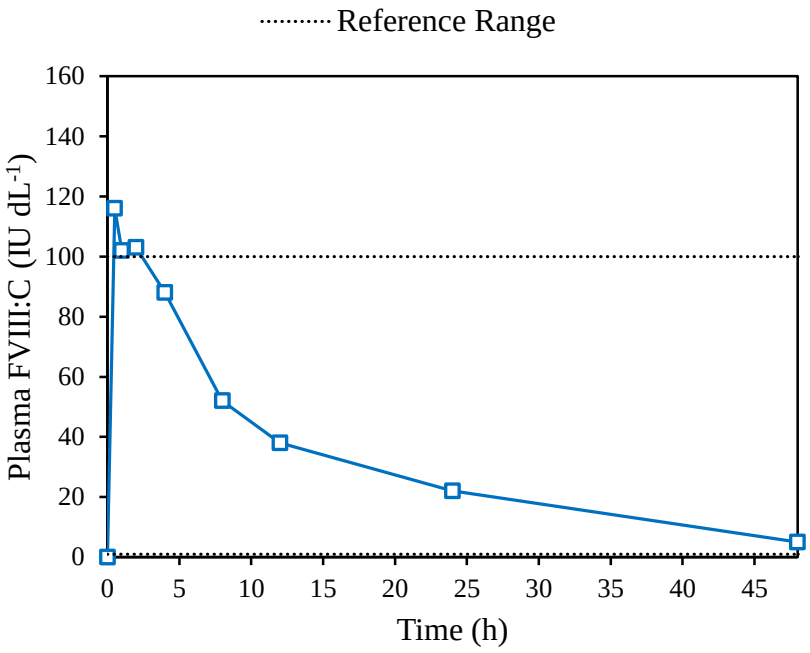


MA-Time Profile

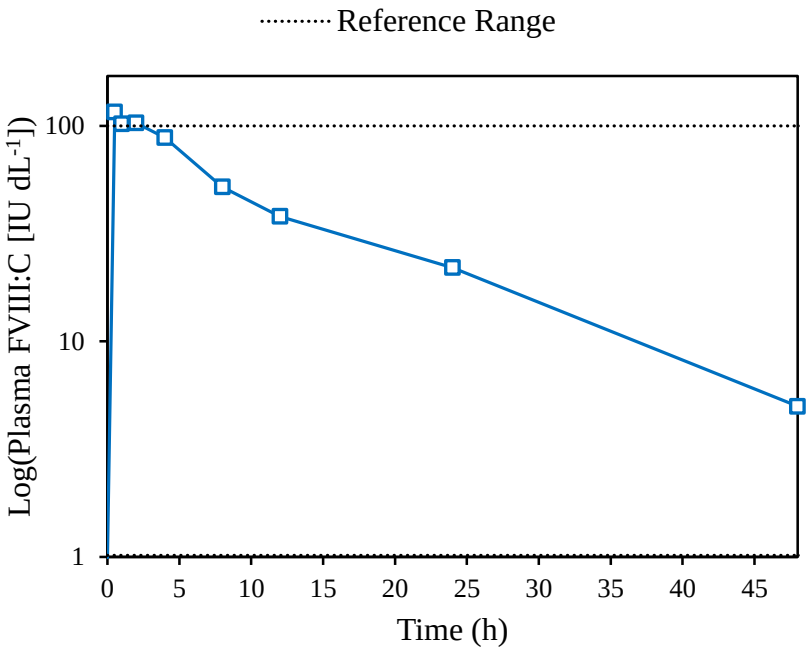


Appendix 8. Individual Profiles for Patient 4

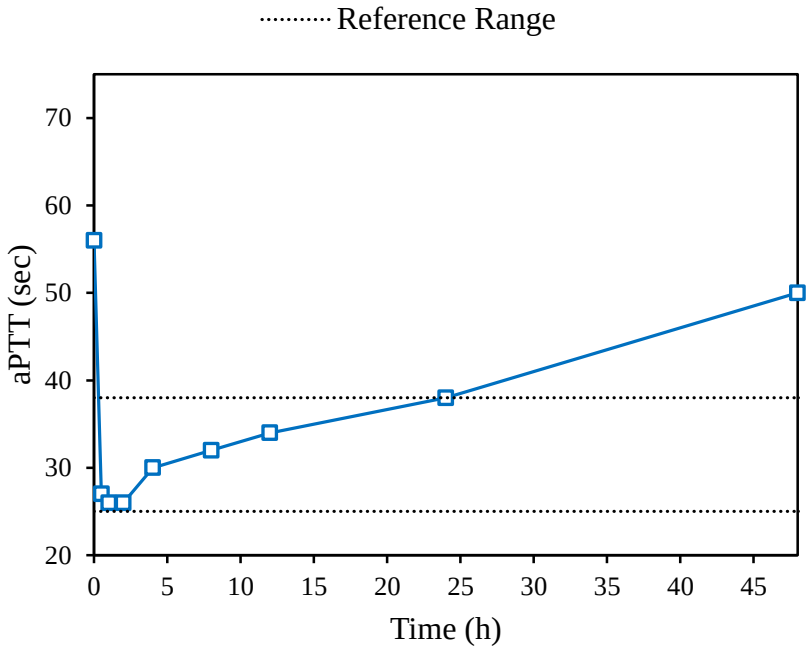
Plasma FVIII:C-Time Profiles



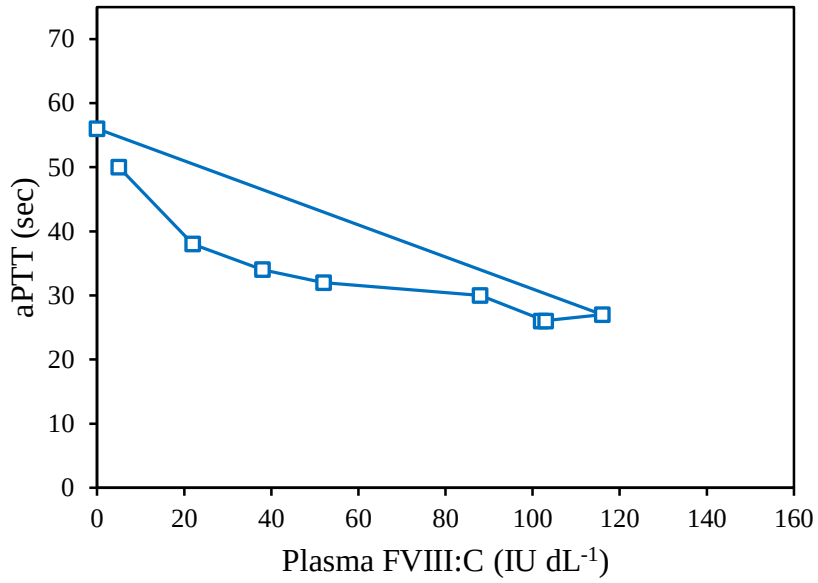
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



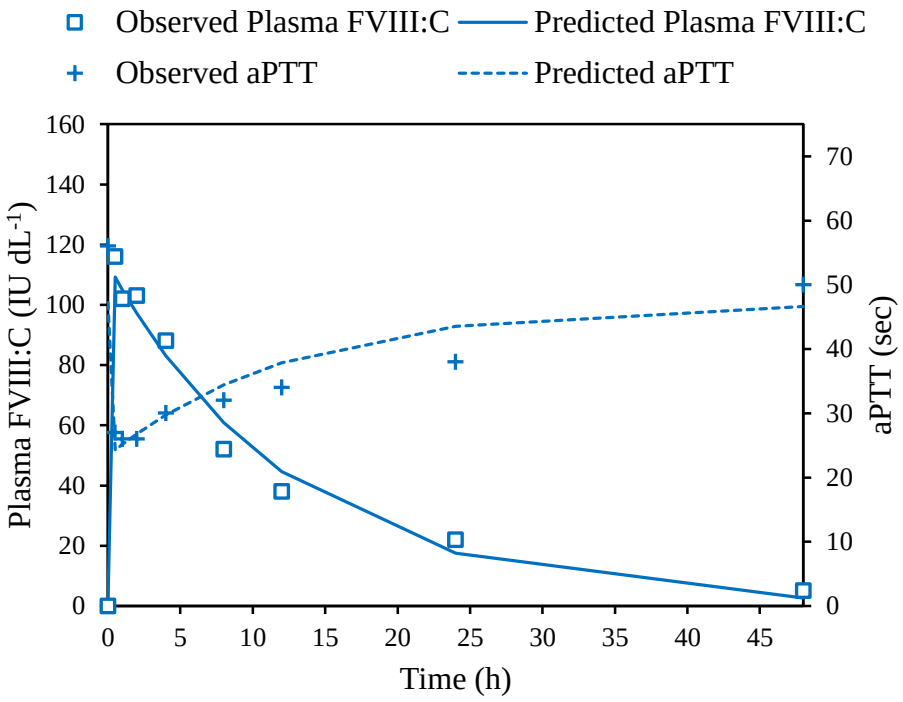
aPTT-Time Profile



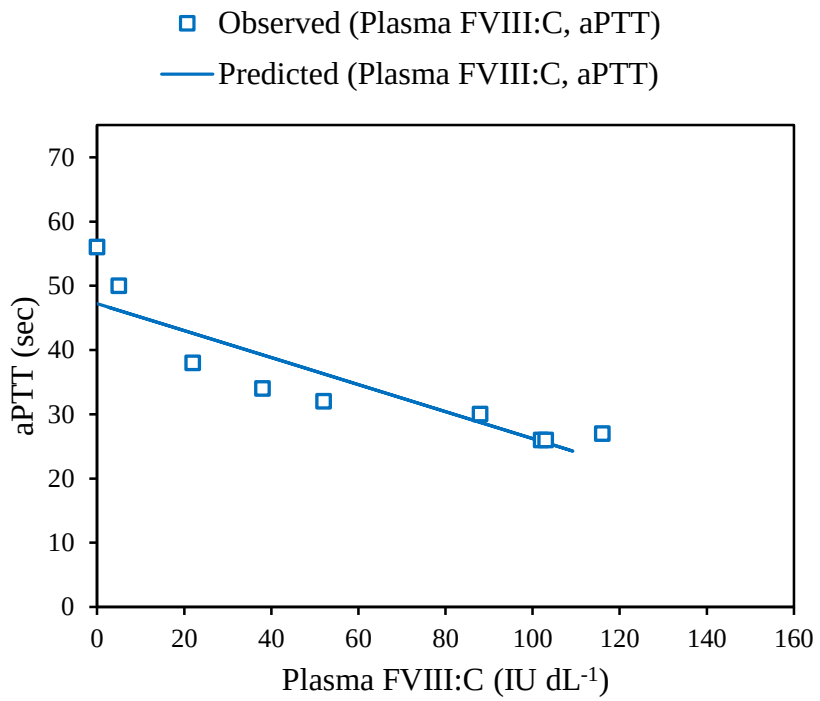
aPTT-Plasma FVIII:C Profile



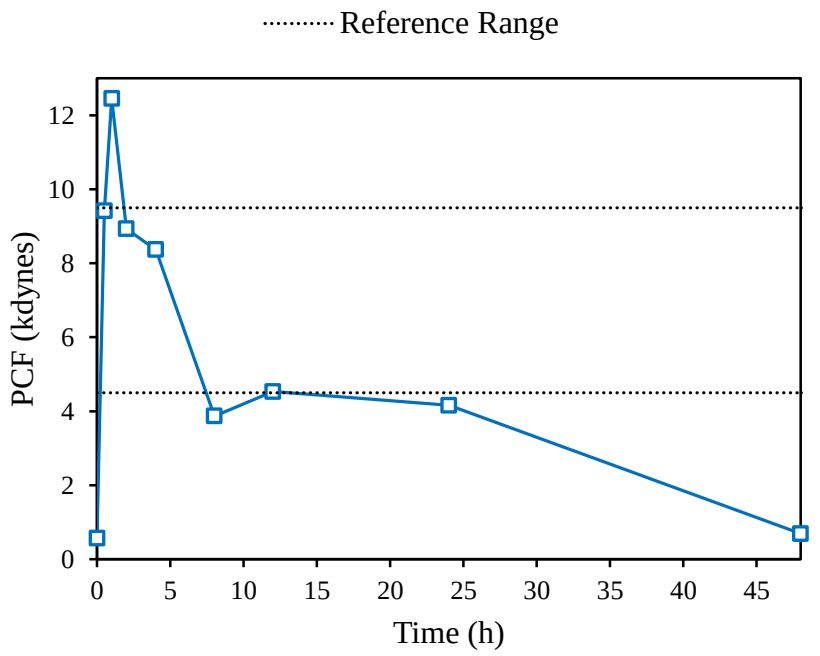
aPTT PK/PD Linear Model Time Dependent Profile



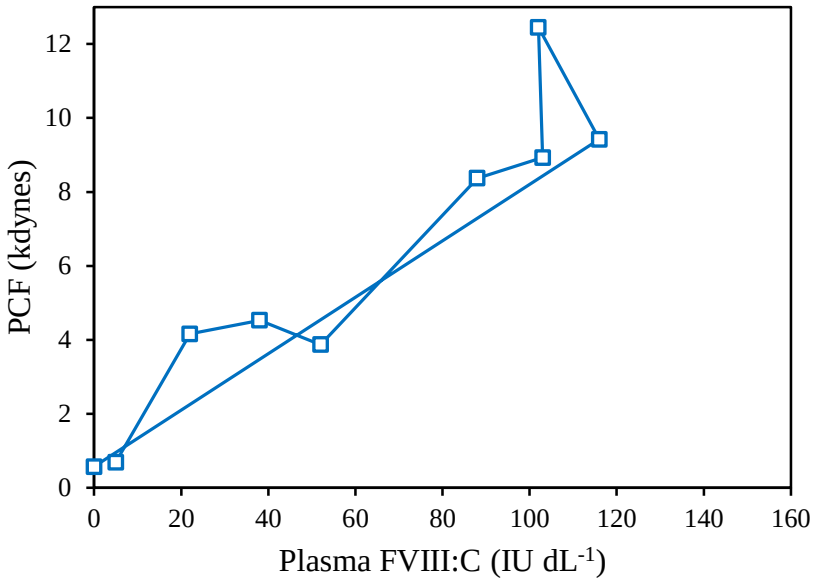
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



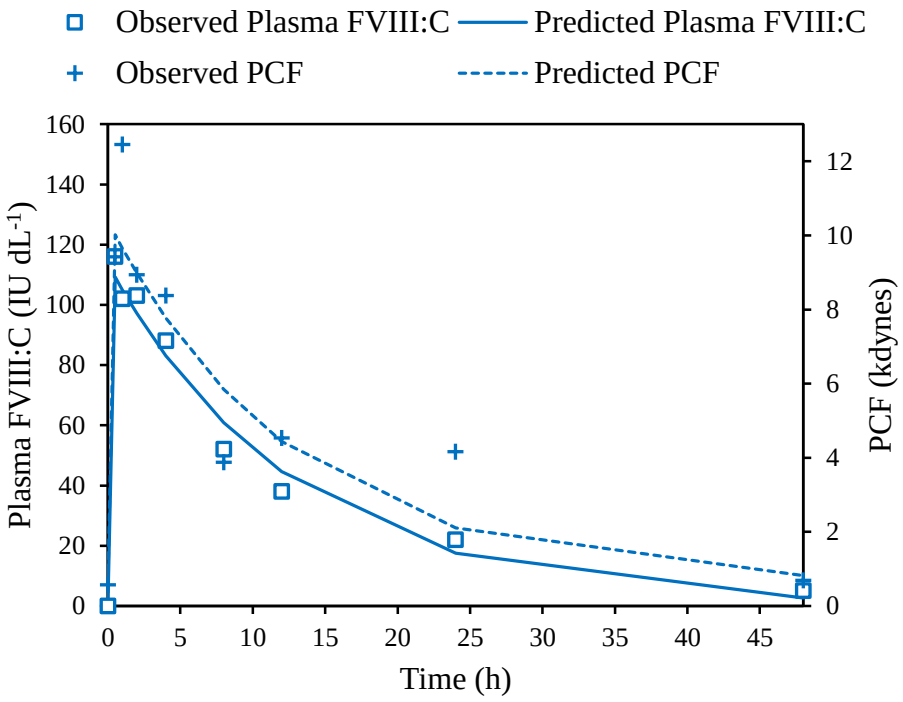
PCF-Time Profile



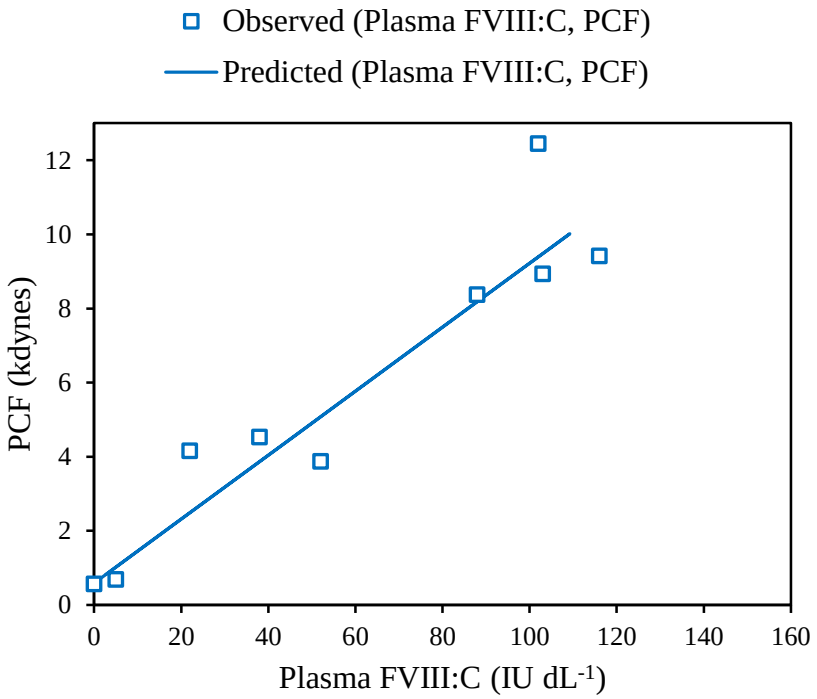
PCF-Plasma FVIII:C Profile



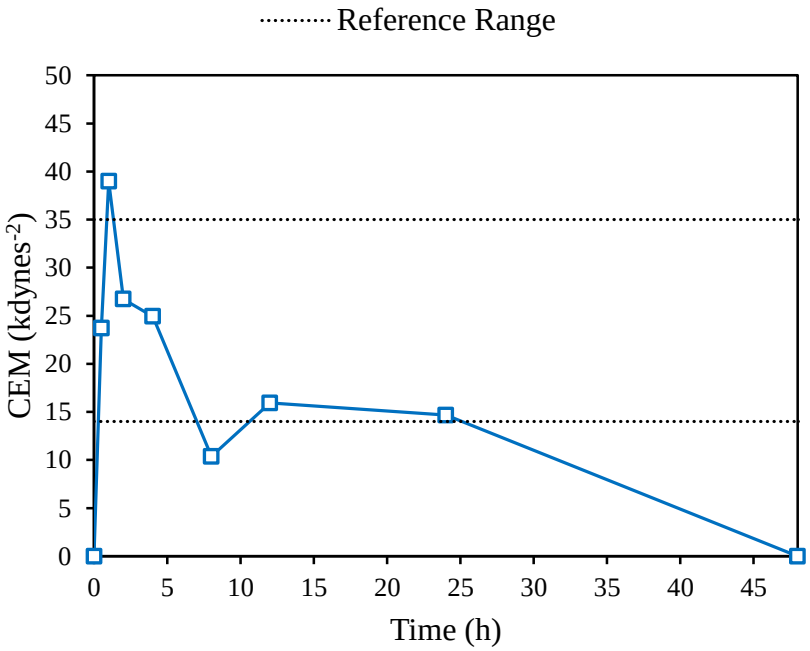
PCF PK/PD Linear Model Time Dependent Profile



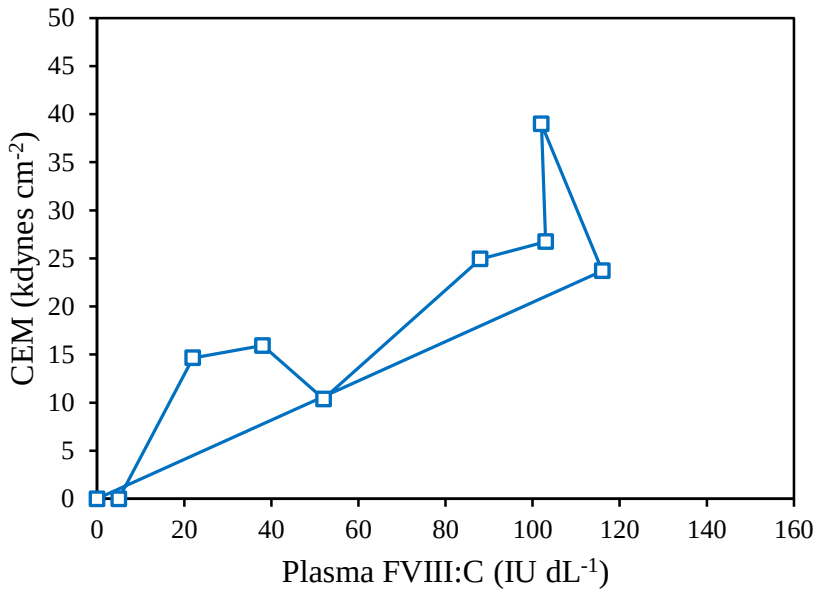
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



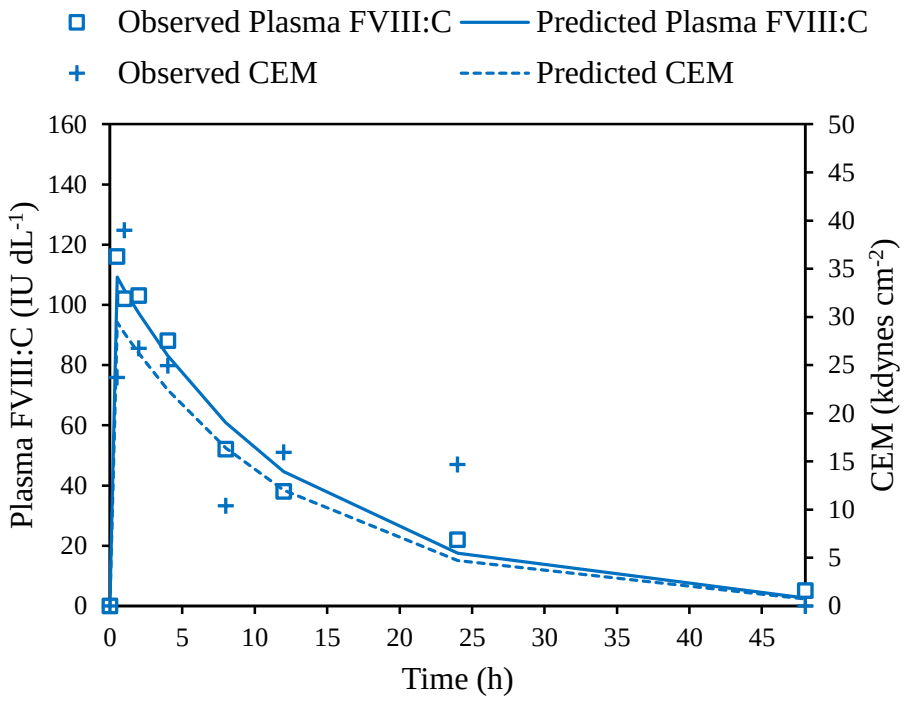
CEM-Time Profile



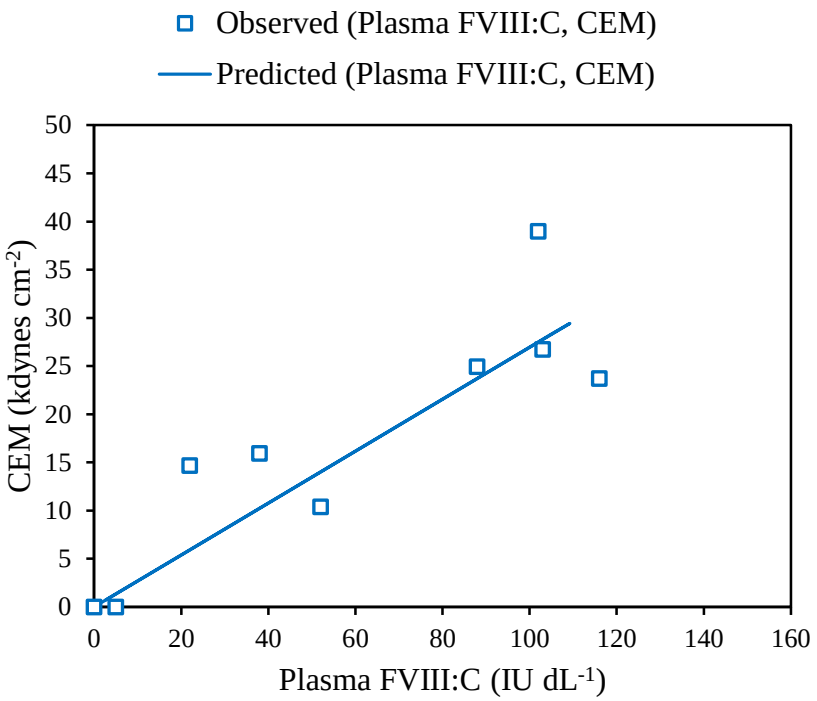
CEM-Plasma FVIII:C Profile



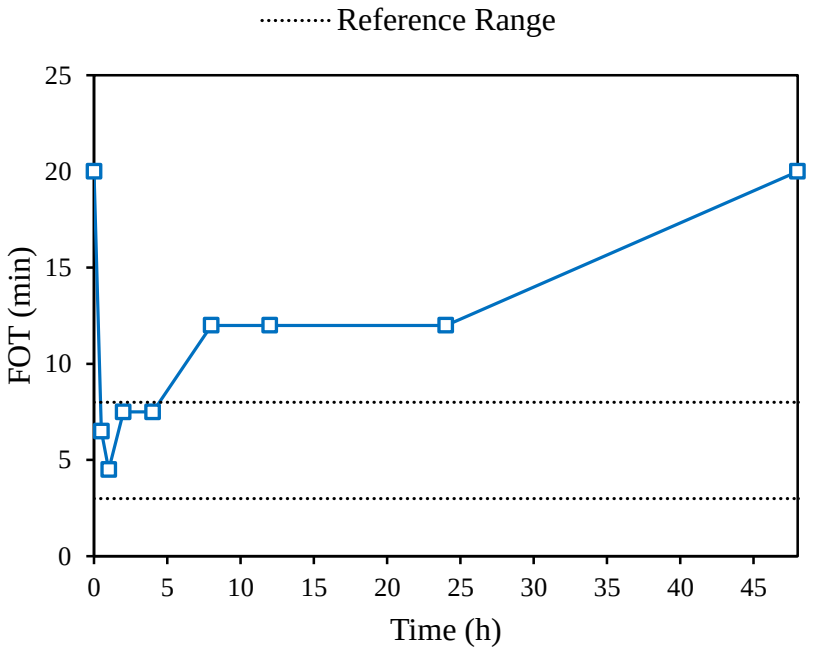
CEM PK/PD Linear Model Time Dependent Profile



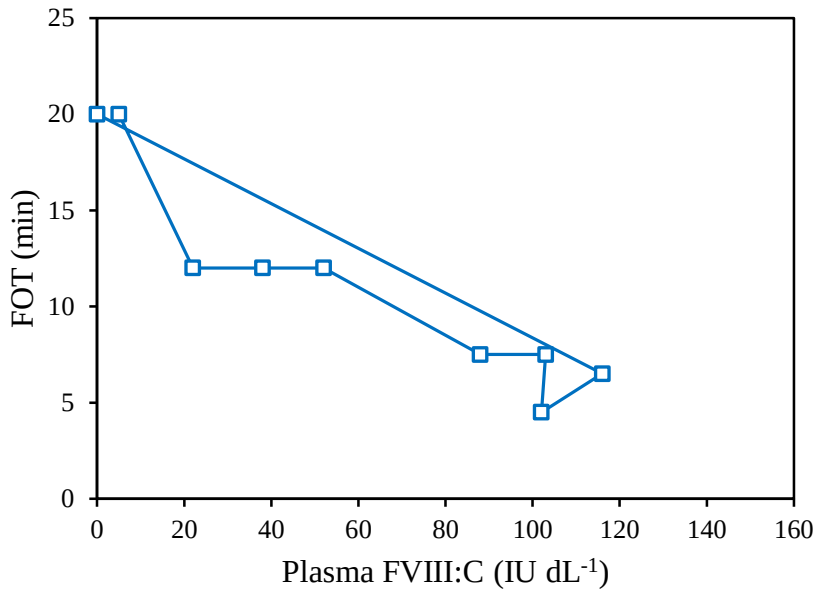
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



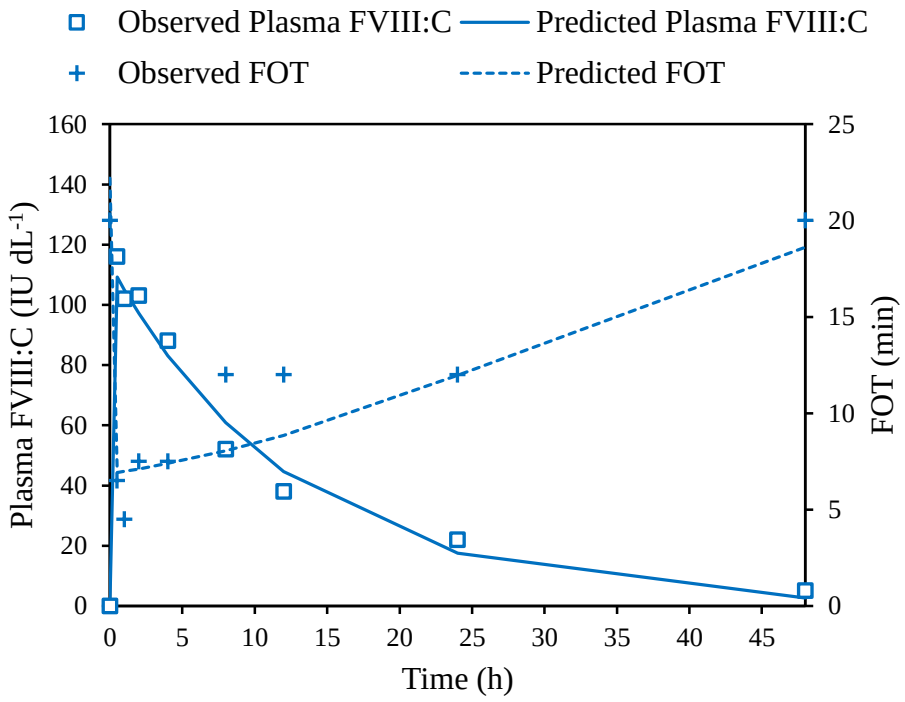
FOT-Time Profile



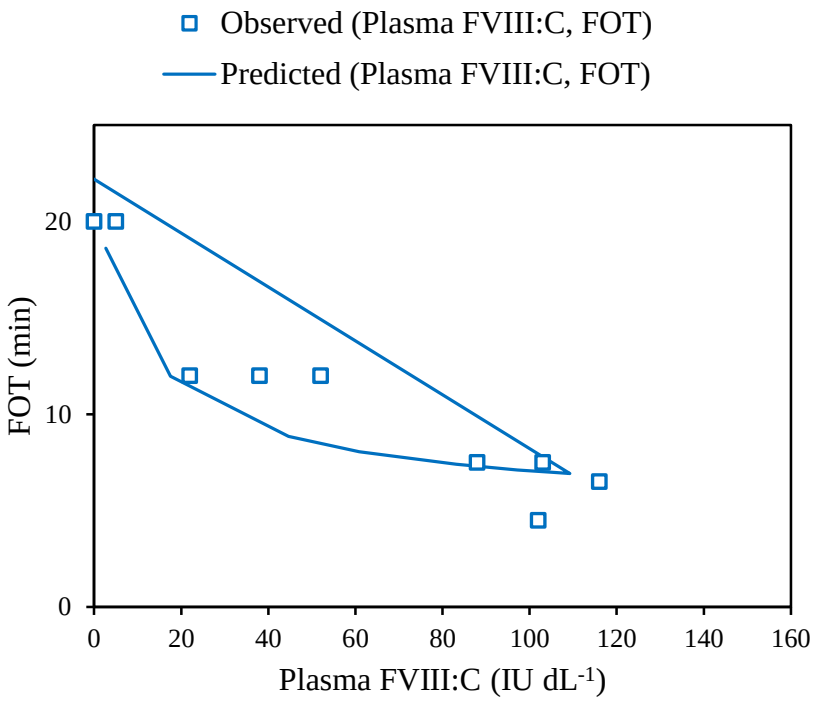
FOT-Plasma FVIII:C Profile



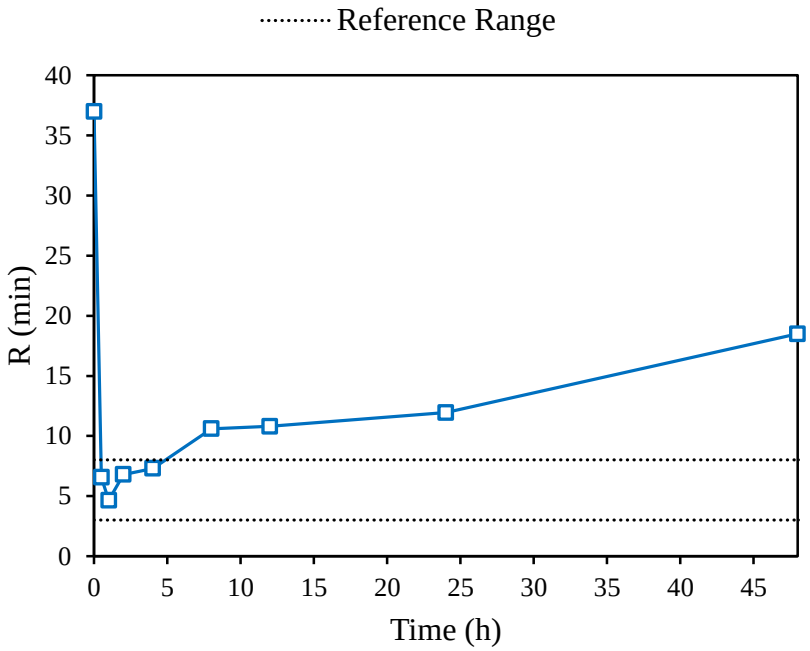
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



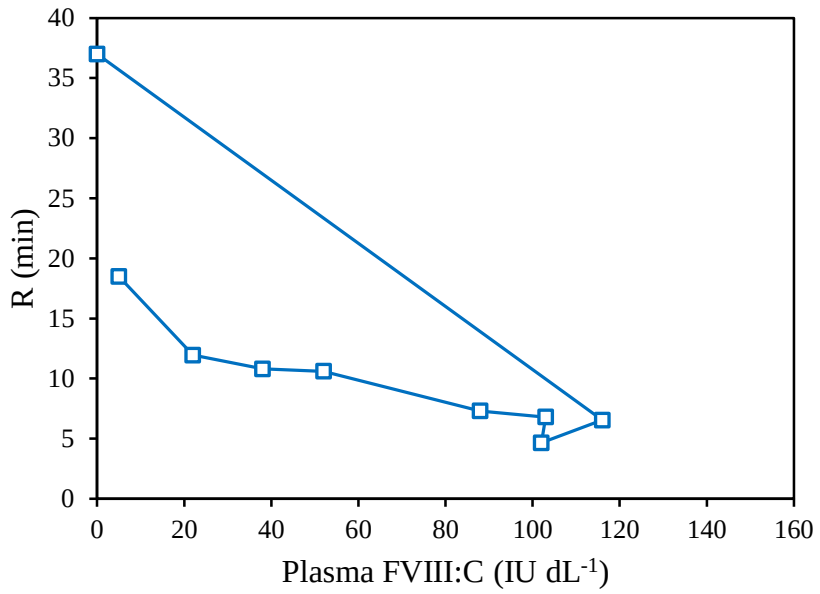
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



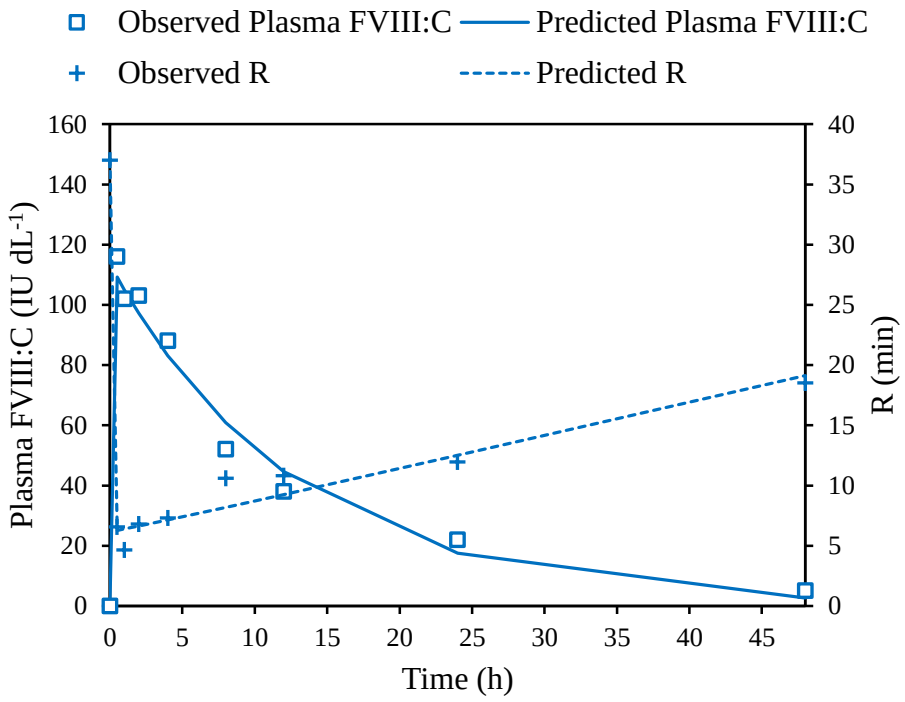
R-Time Profile



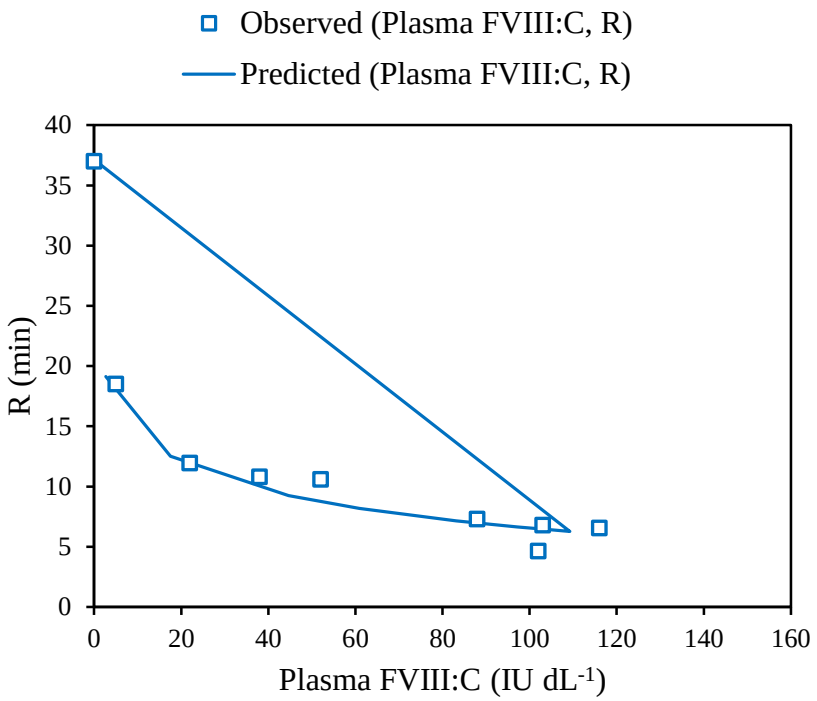
R-Plasma FVIII:C Profile



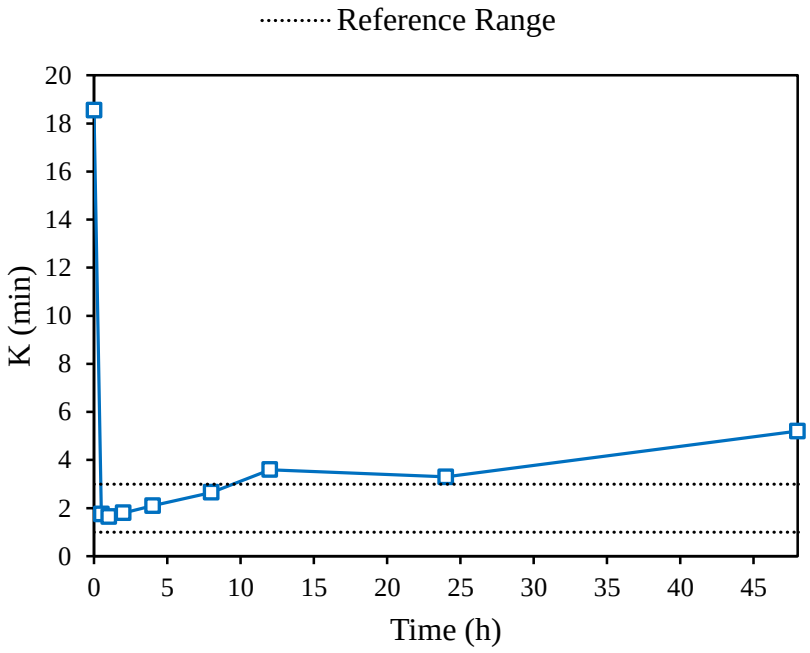
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



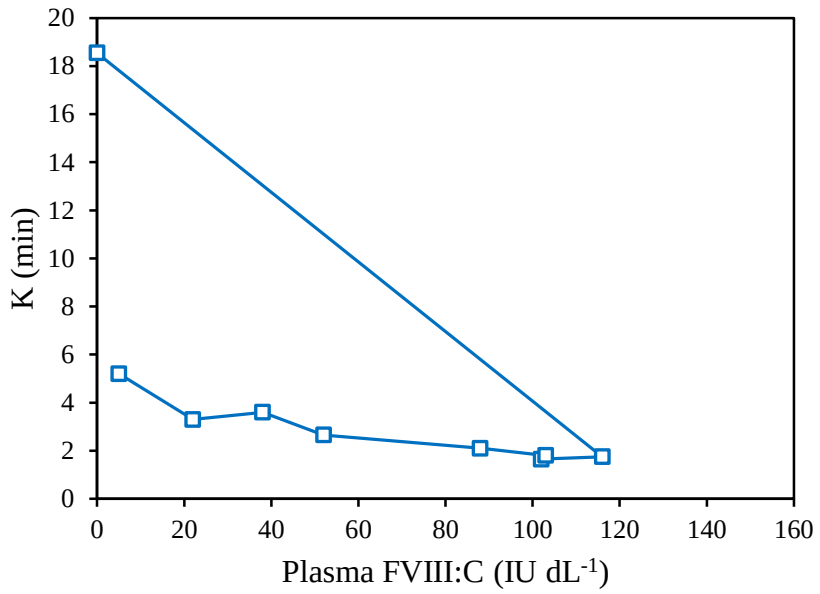
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



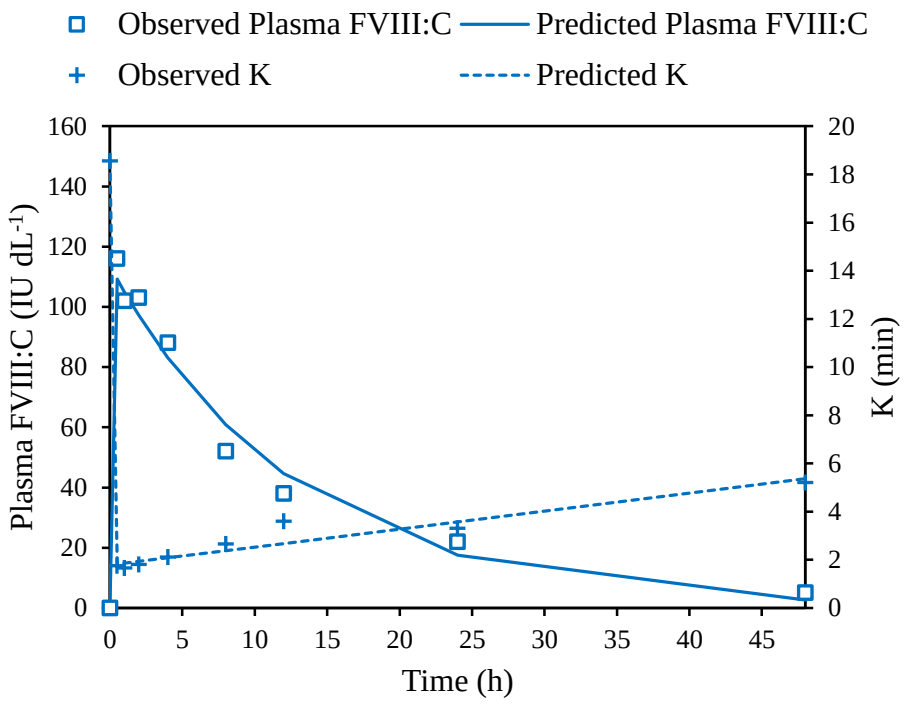
K-Time Profile



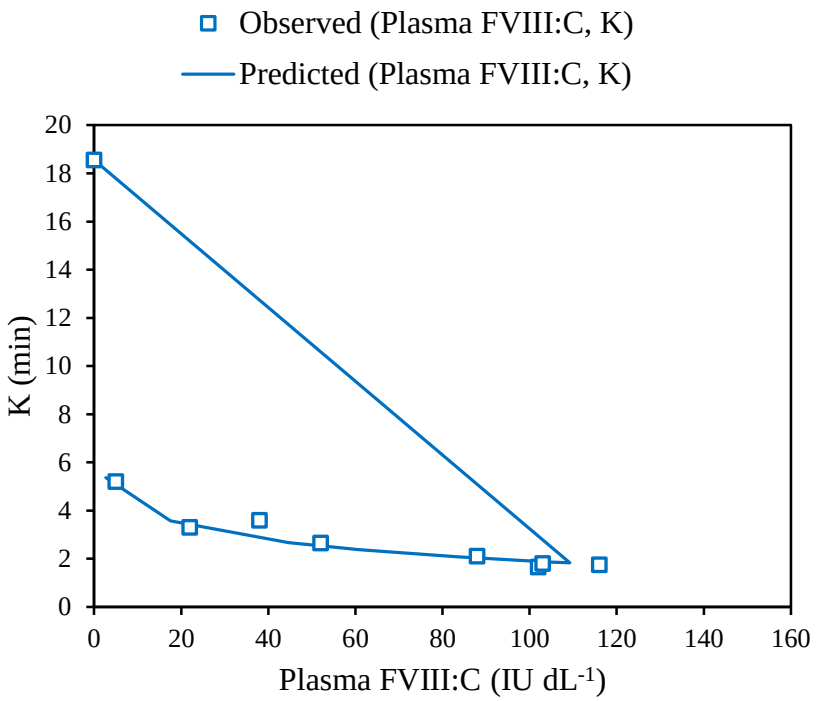
K-Plasma FVIII:C Profile



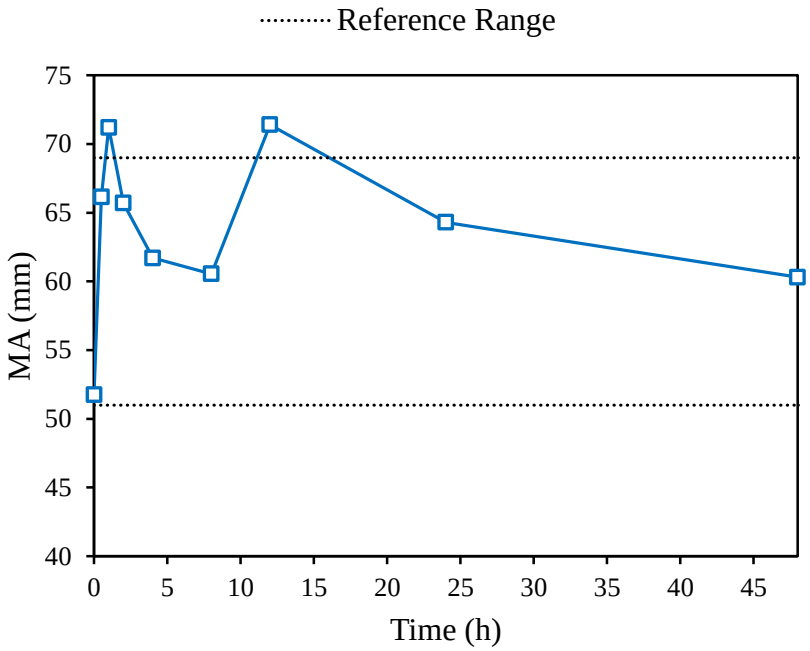
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

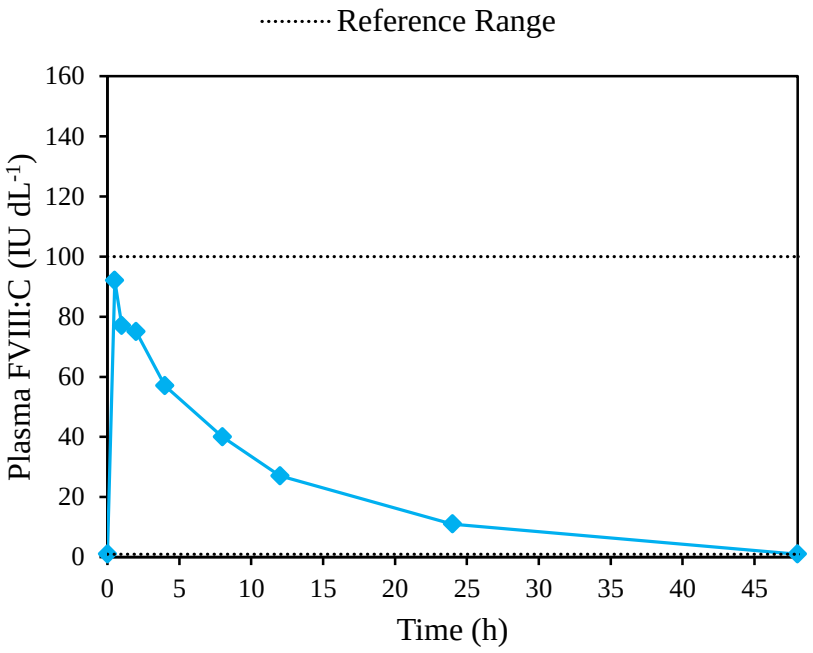


MA-Time Profile

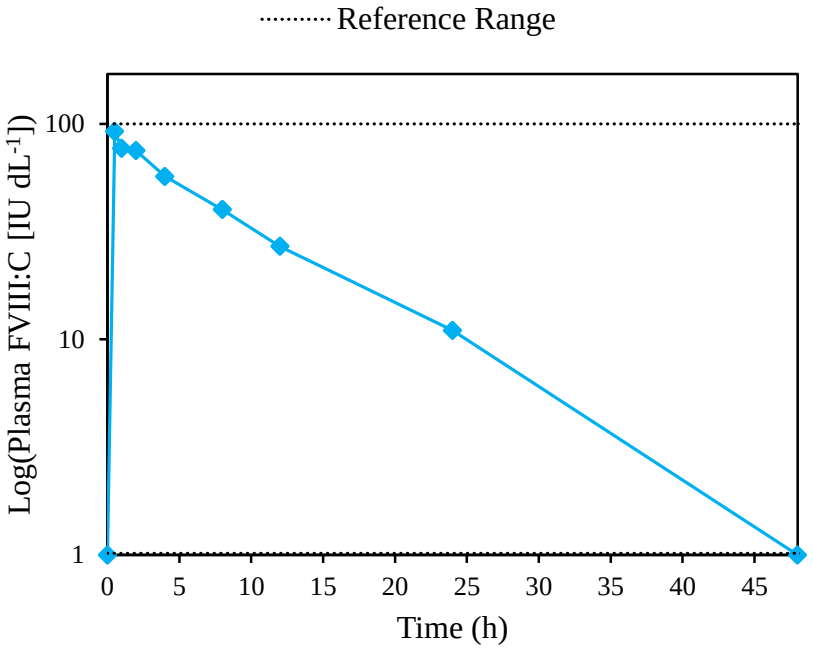


Appendix 9. Individual Profiles for Patient 5

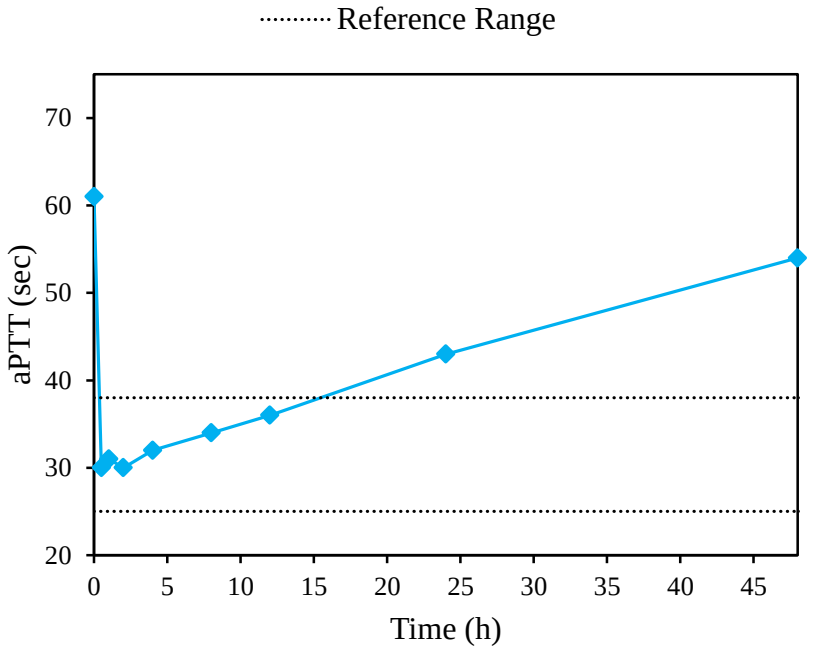
Plasma FVIII:C-Time Profiles



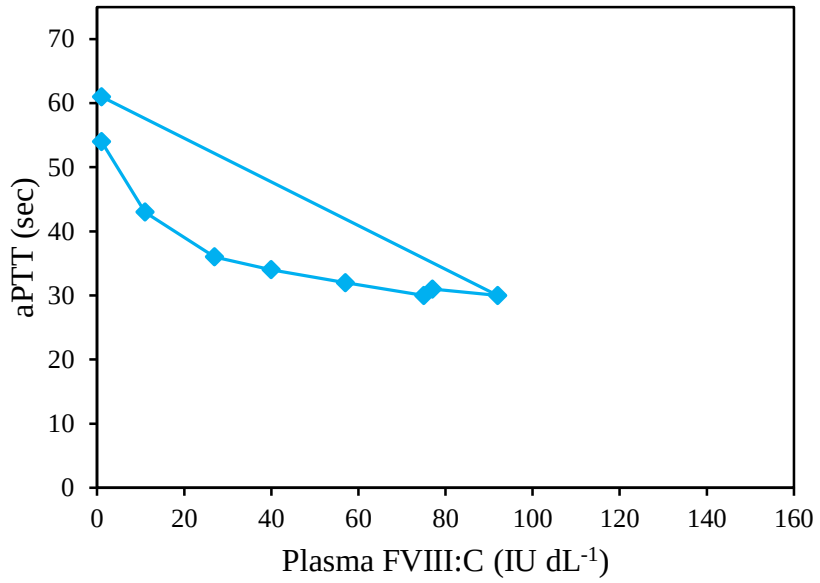
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



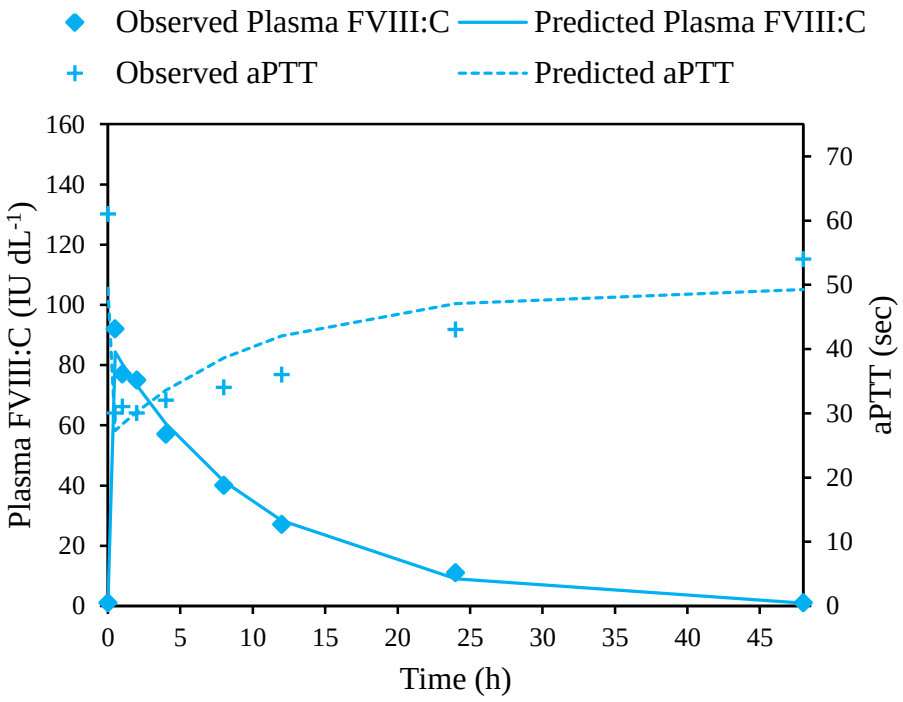
aPTT-Time Profile



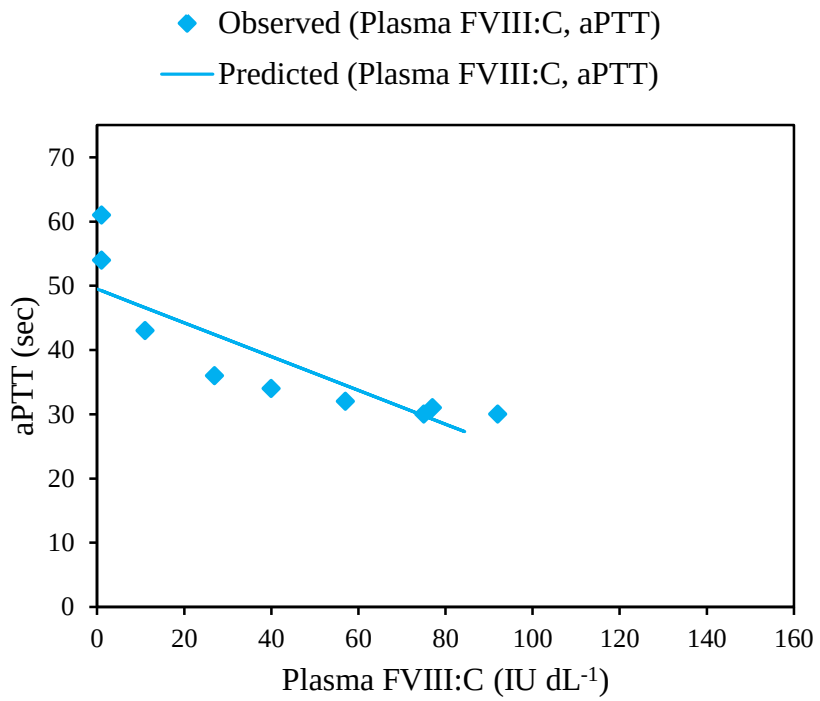
aPTT-Plasma FVIII:C Profile



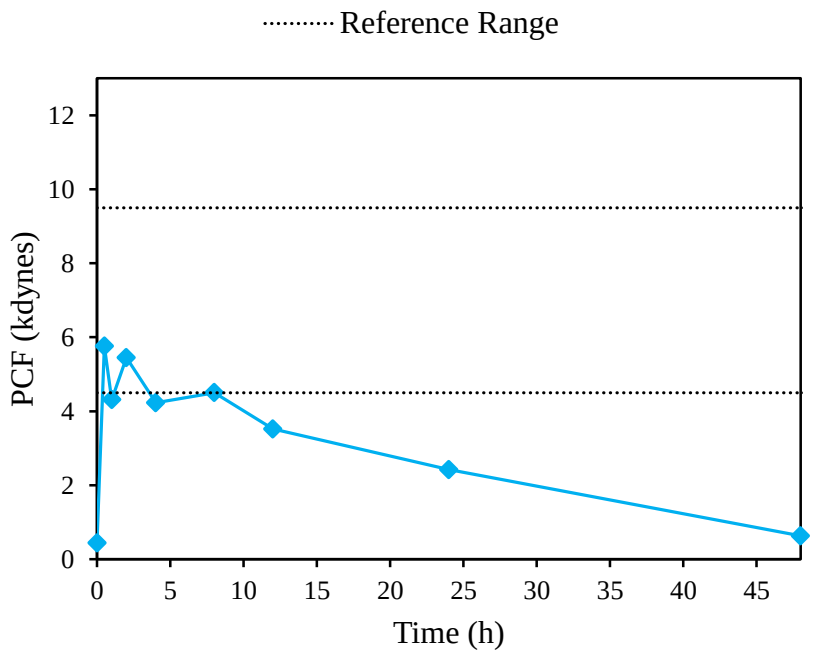
aPTT PK/PD Linear Model Time Dependent Profile



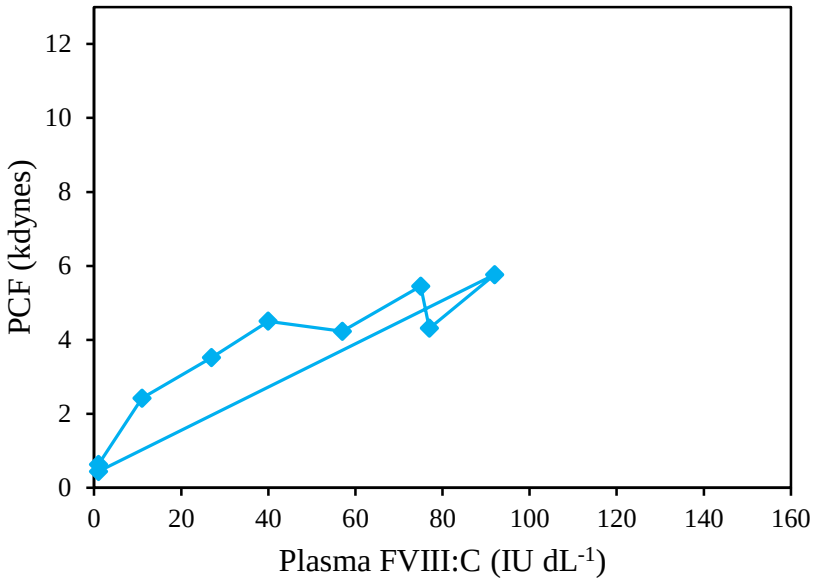
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



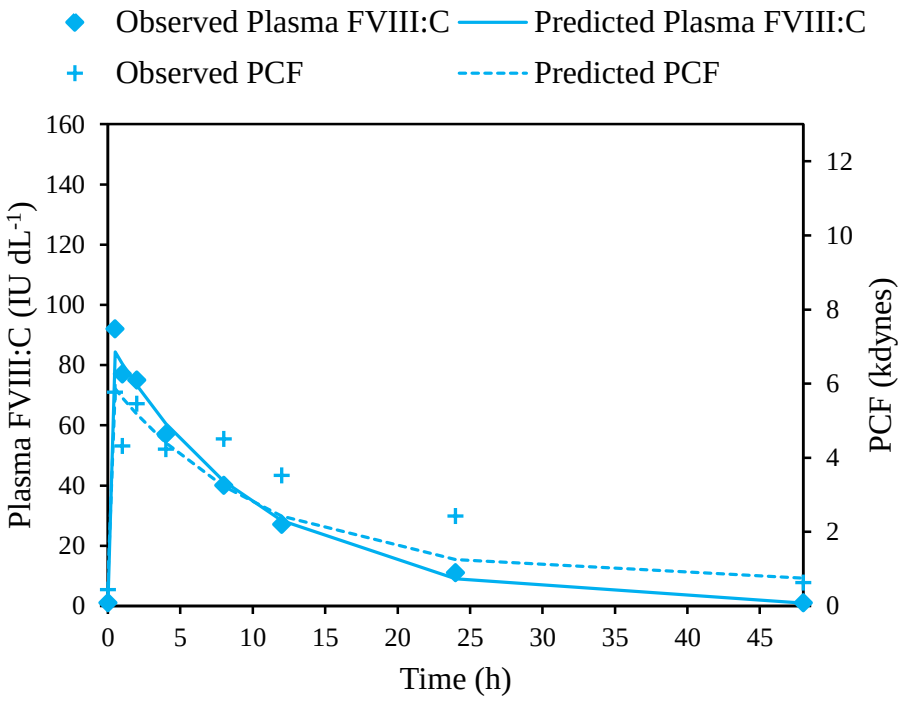
PCF-Time Profile



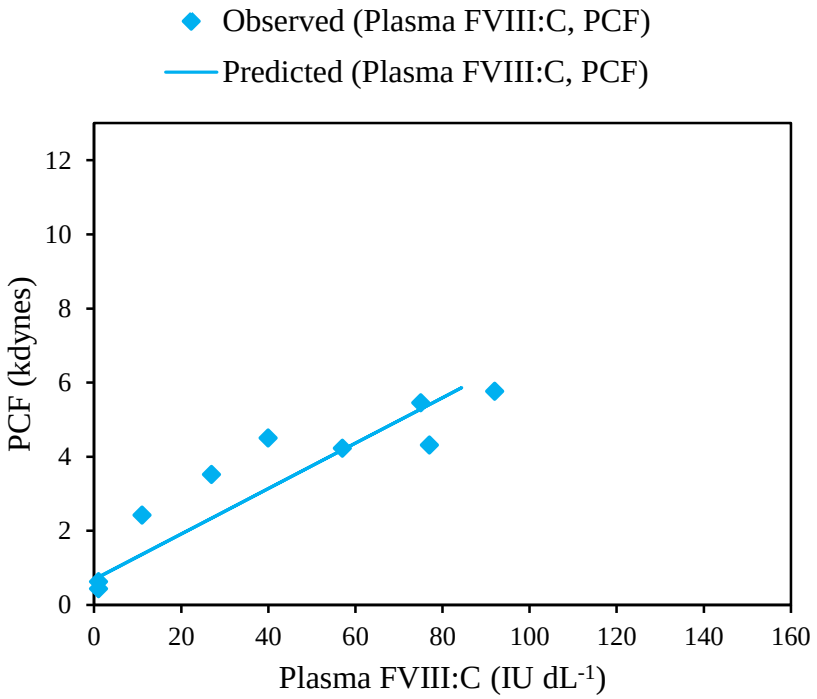
PCF-Plasma FVIII:C Profile



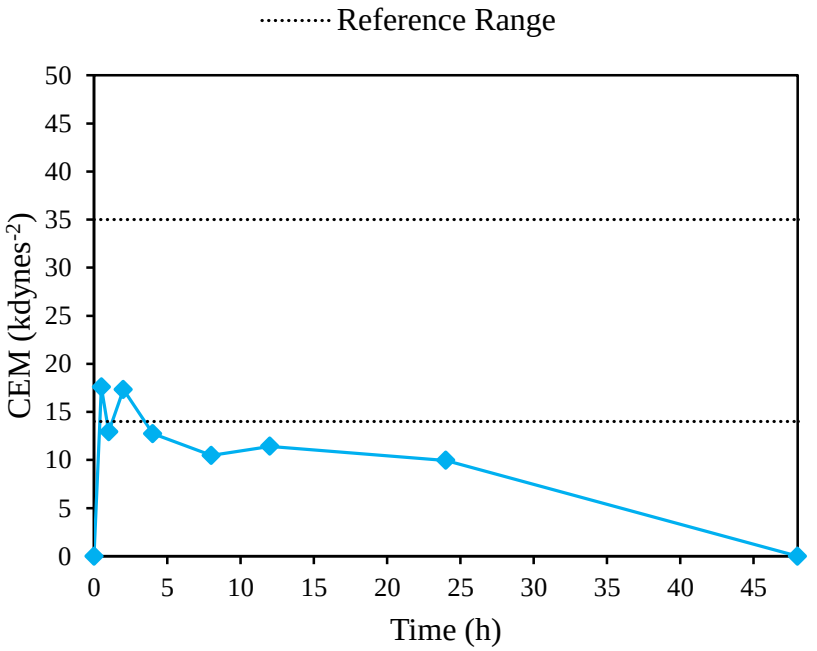
PCF PK/PD Linear Model Time Dependent Profile



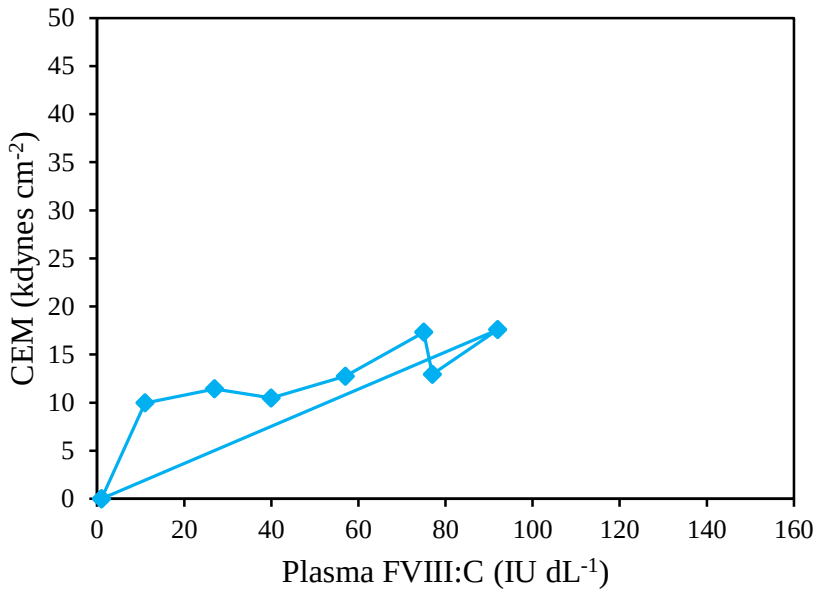
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



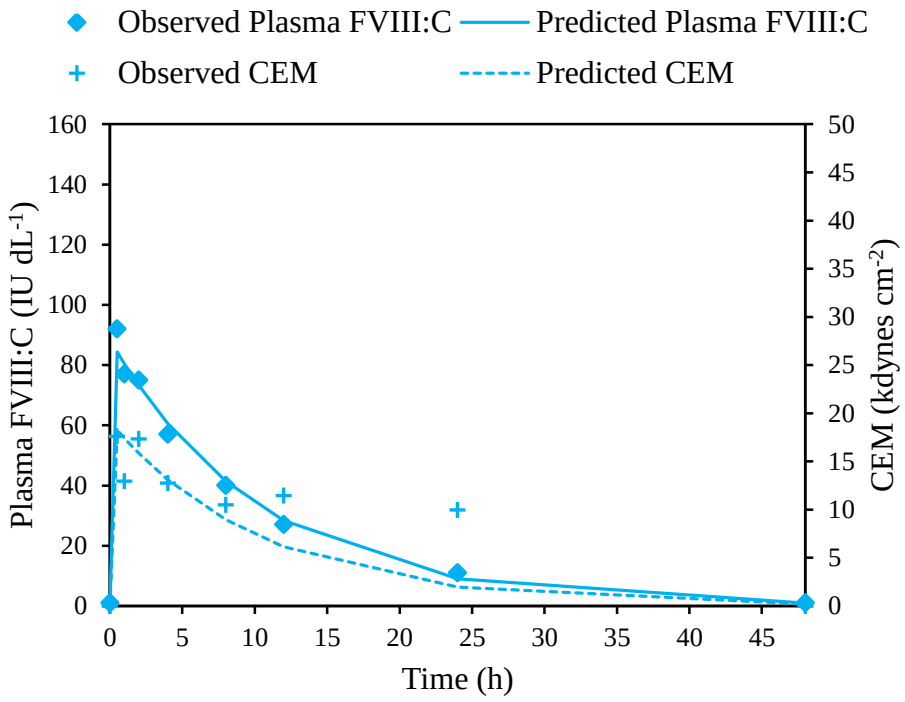
CEM-Time Profile



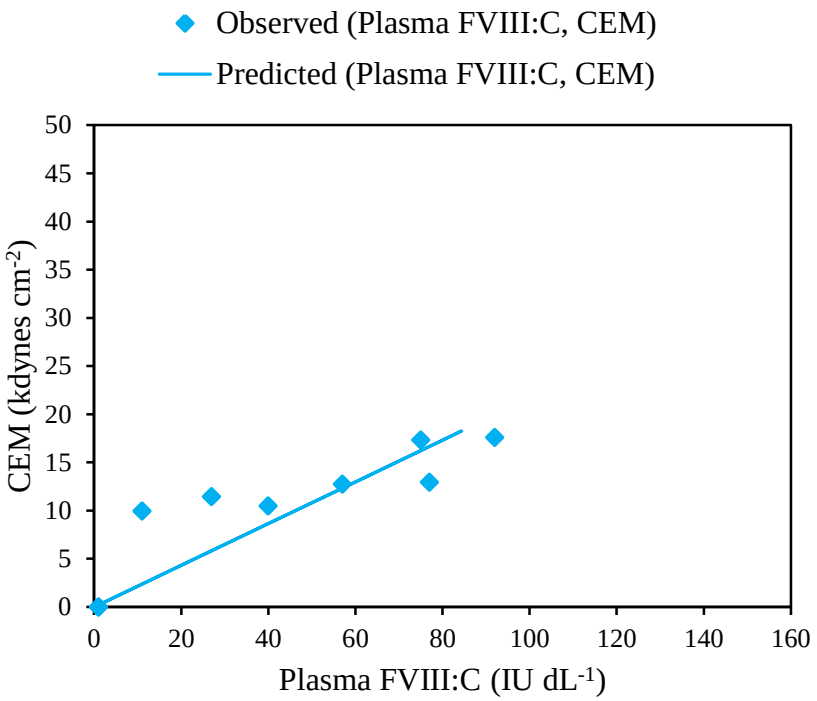
CEM-Plasma FVIII:C Profile



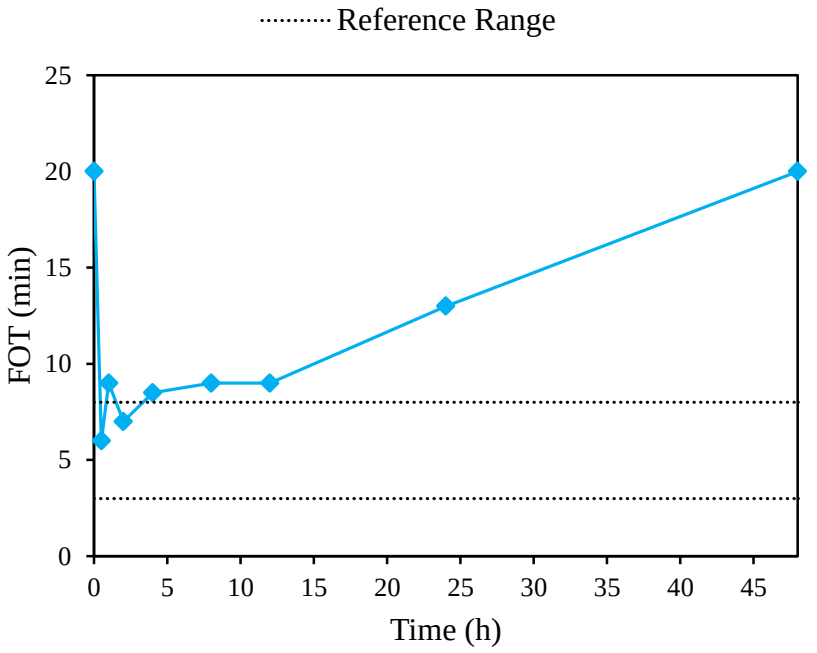
CEM PK/PD Linear Model Time Dependent Profile



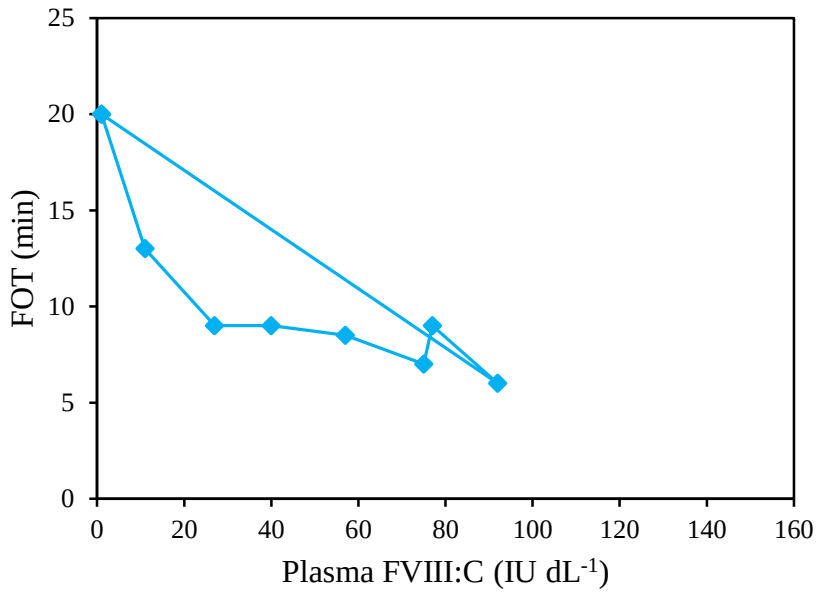
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



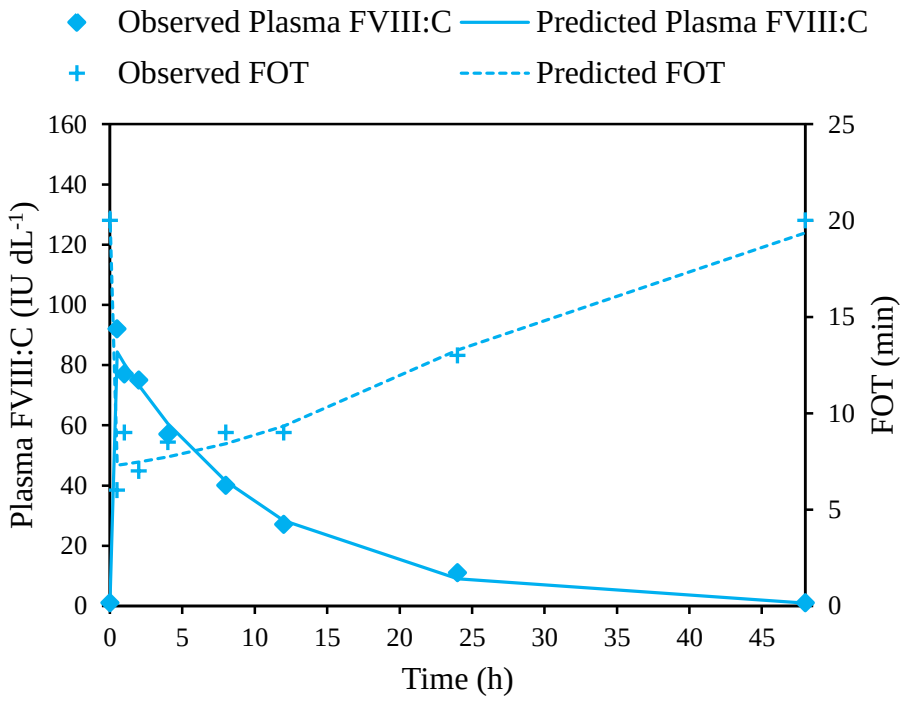
FOT-Time Profile



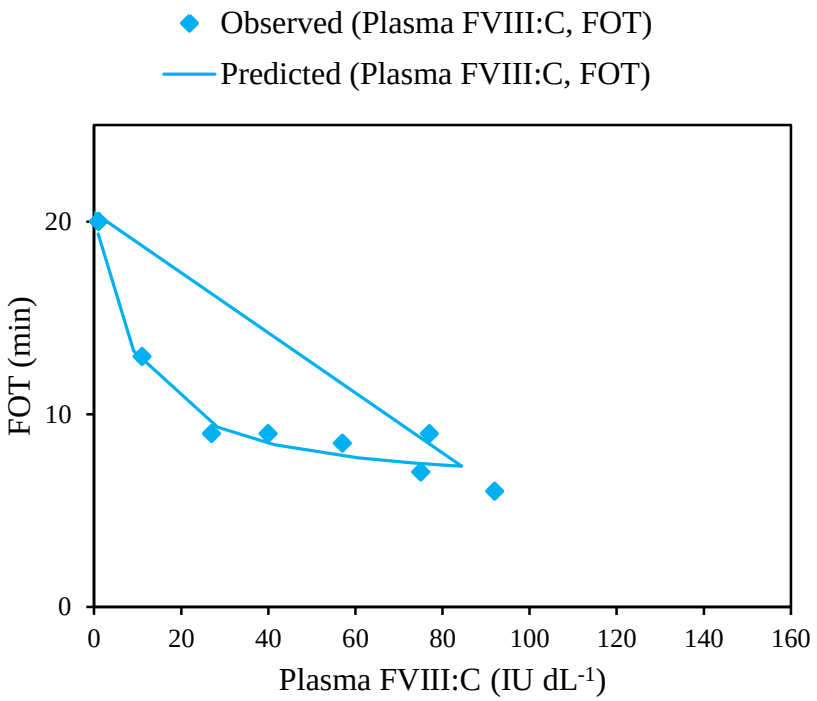
FOT-Plasma FVIII:C Profile



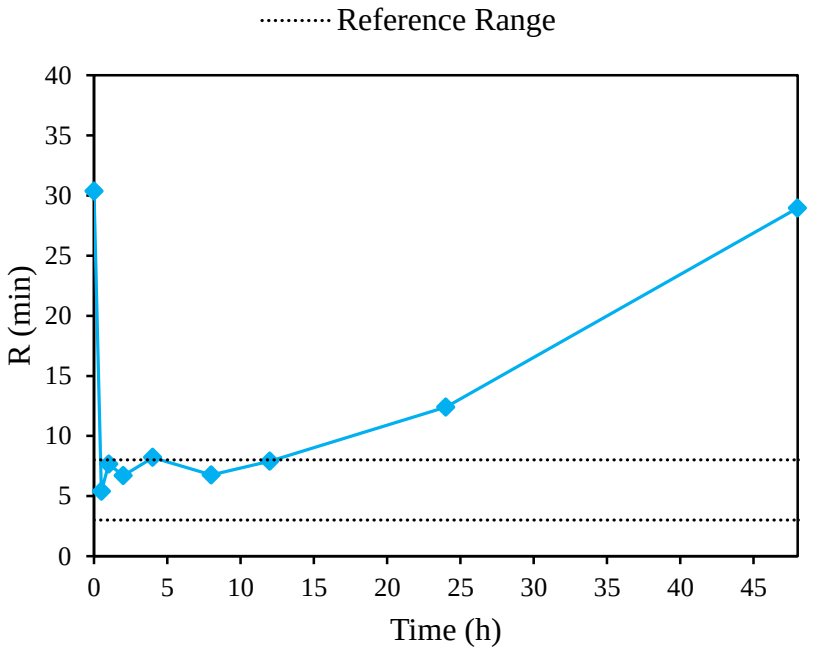
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



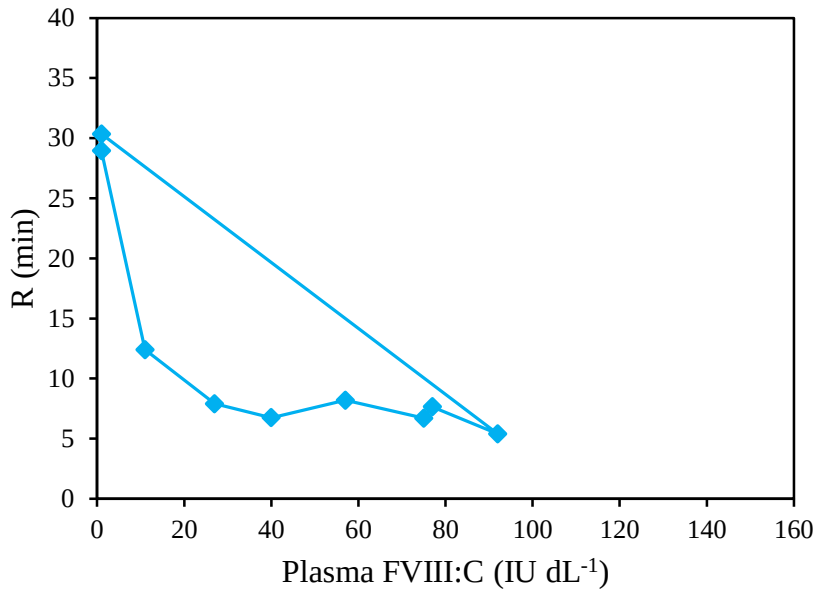
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



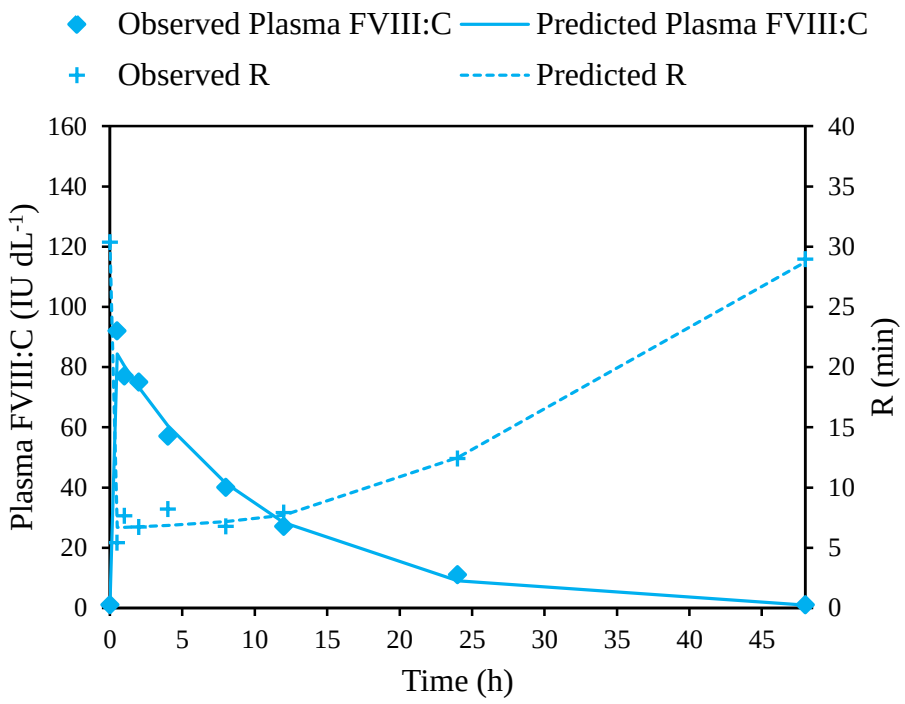
R-Time Profile



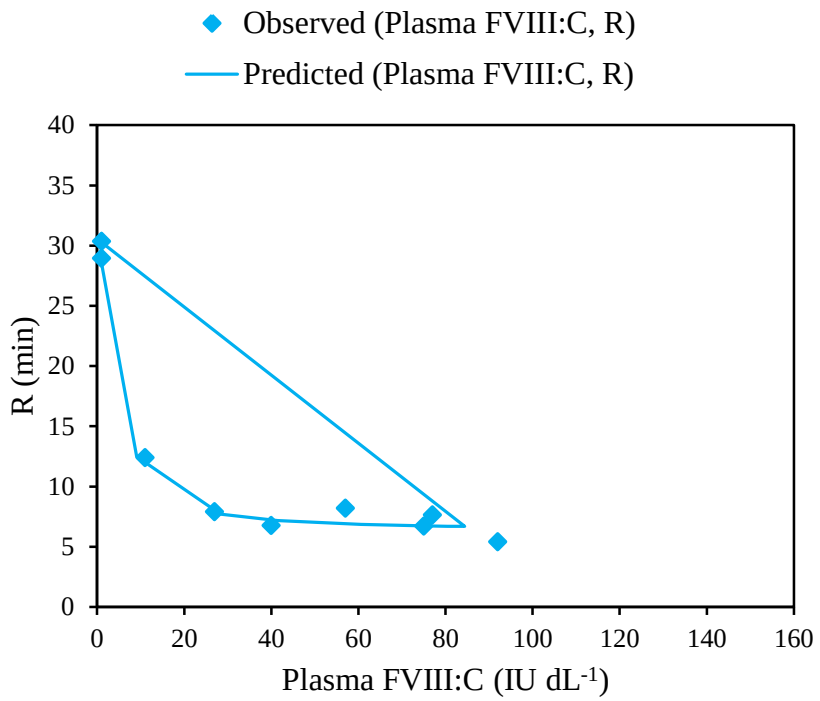
R-Plasma FVIII:C Profile



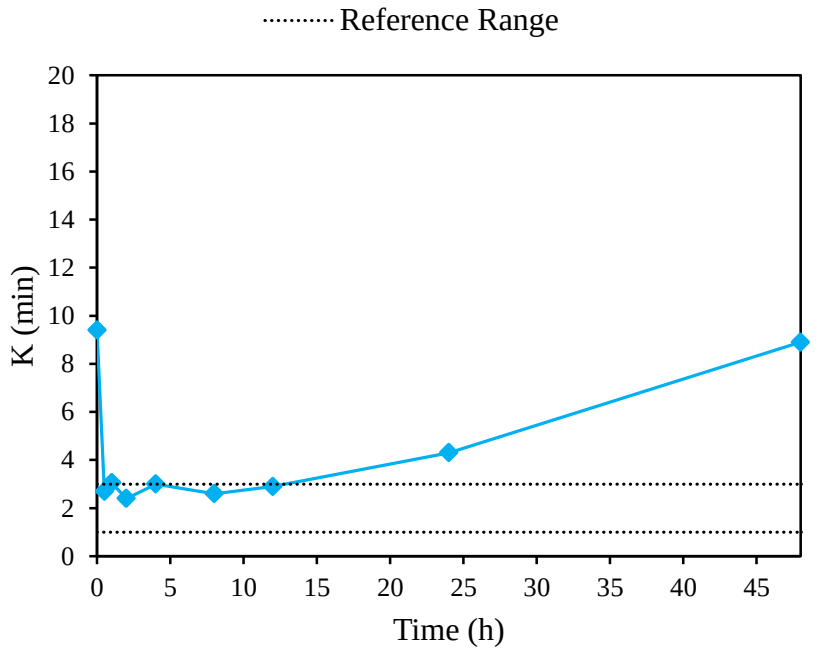
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



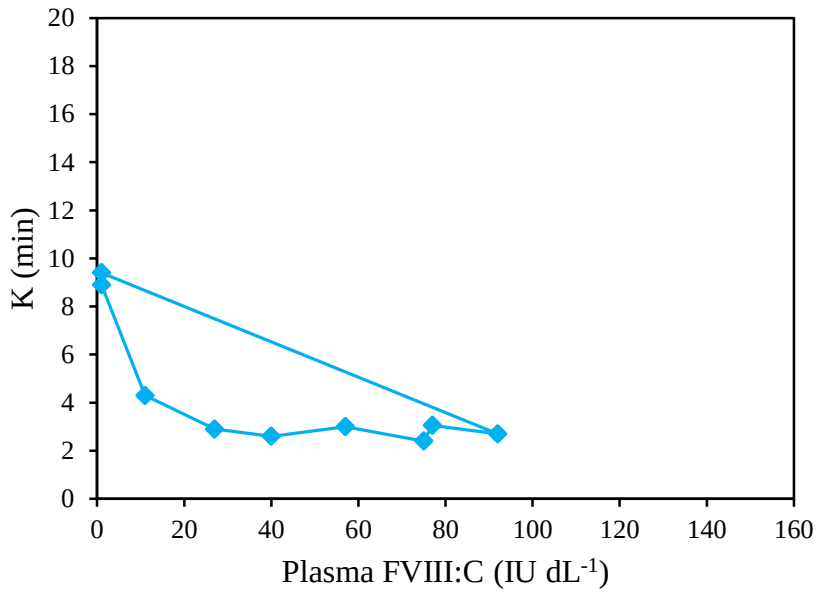
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



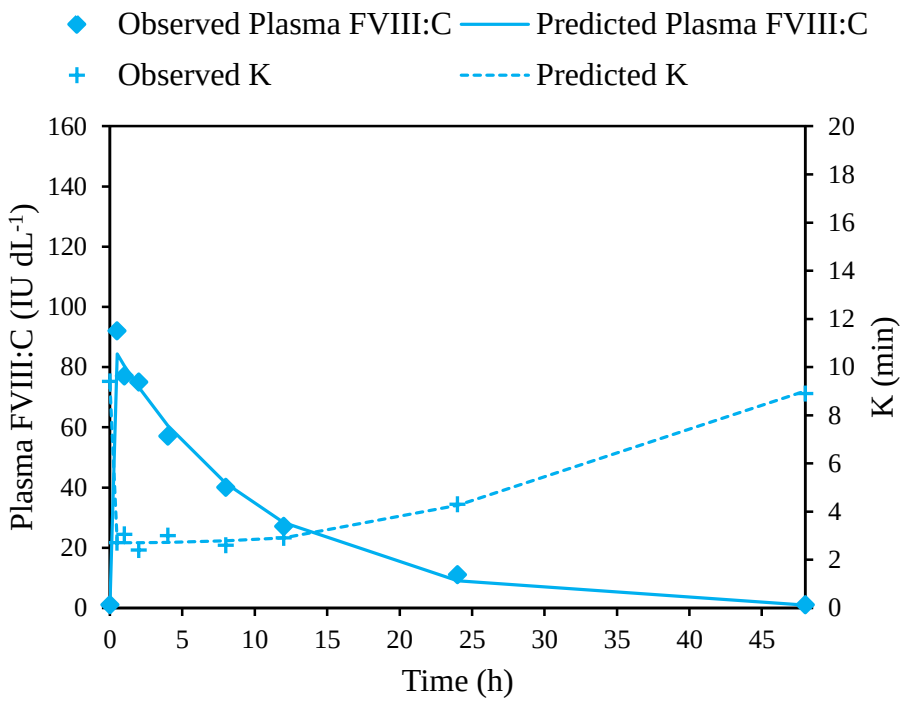
K-Time Profile



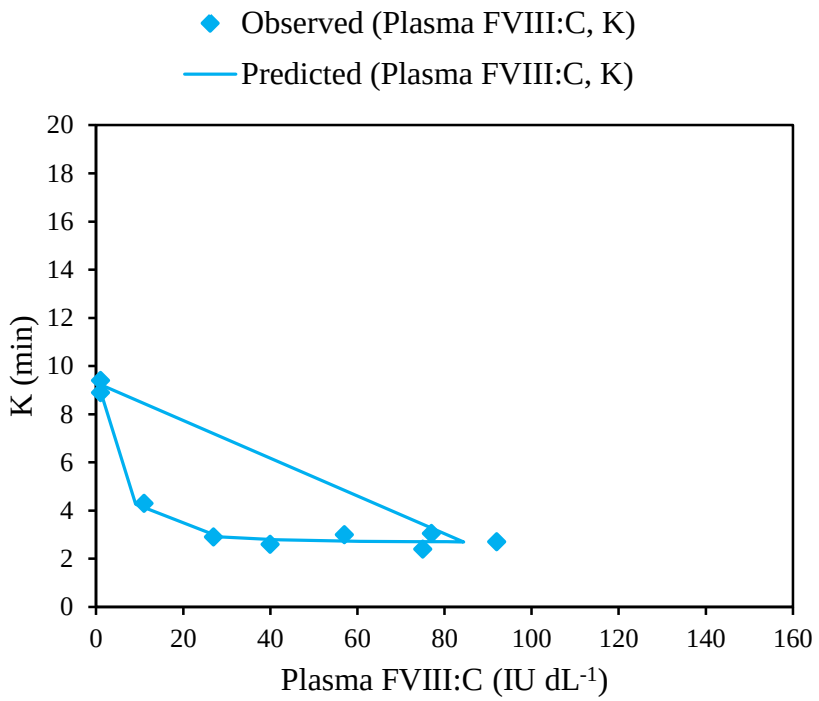
K-Plasma FVIII:C Profile



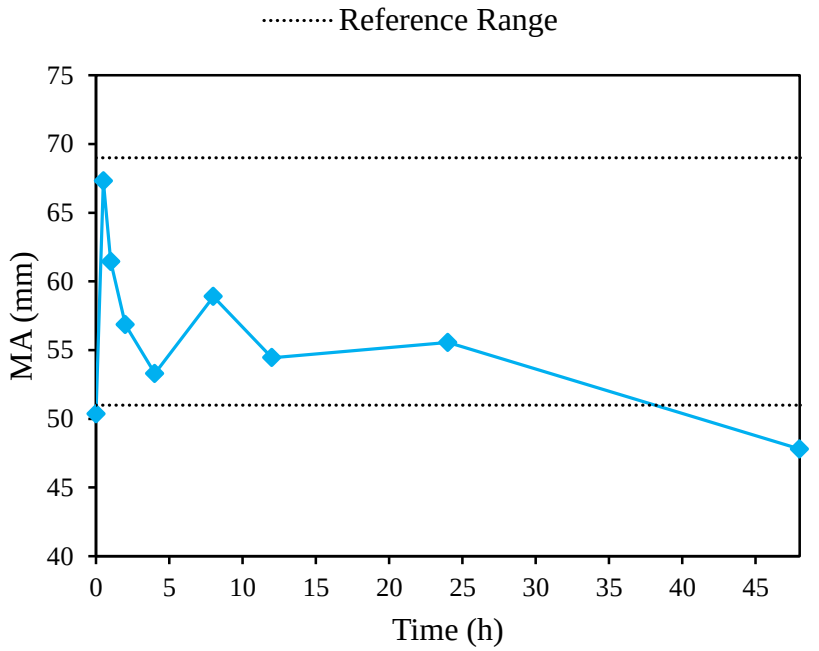
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

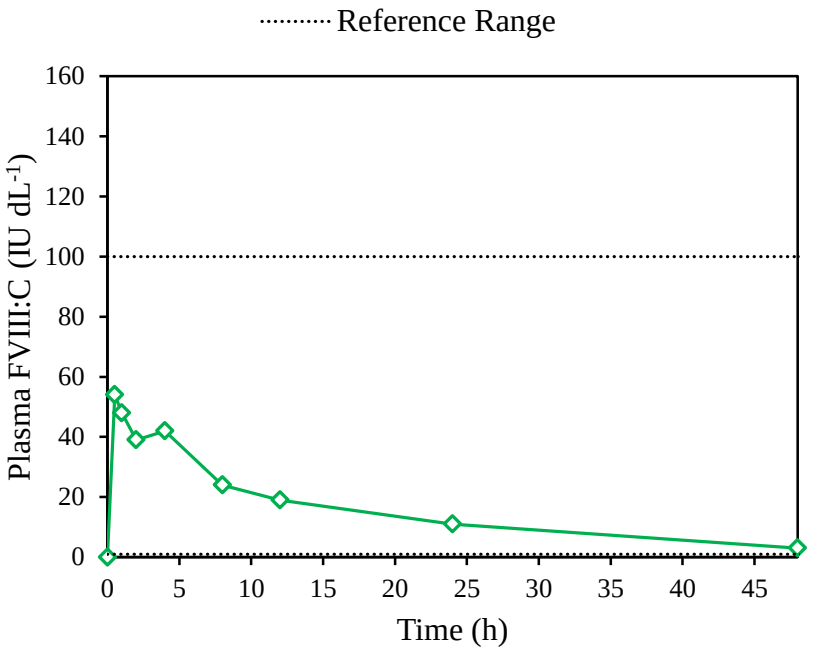


MA-Time Profile

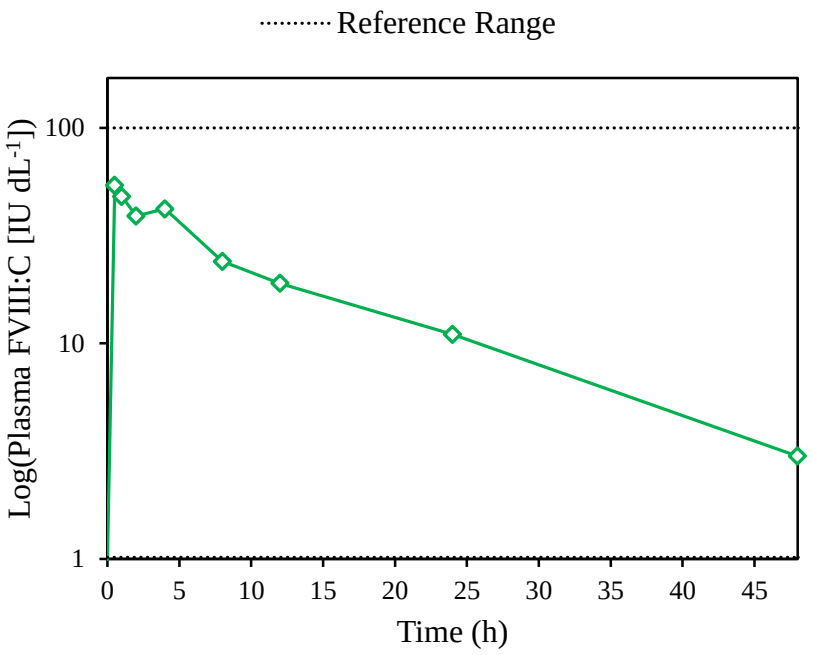


Appendix 10. Individual Profiles for Patient 6

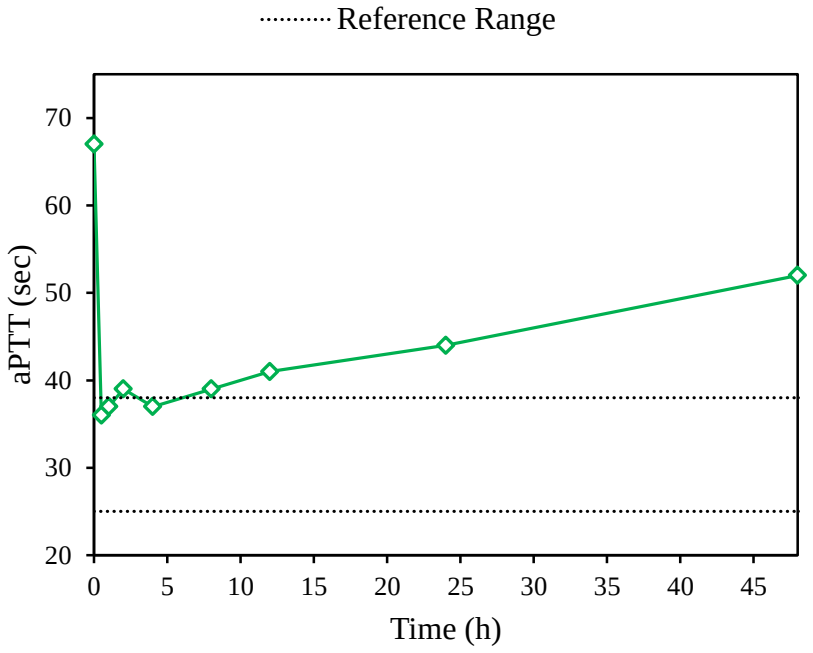
Plasma FVIII:C-Time Profiles



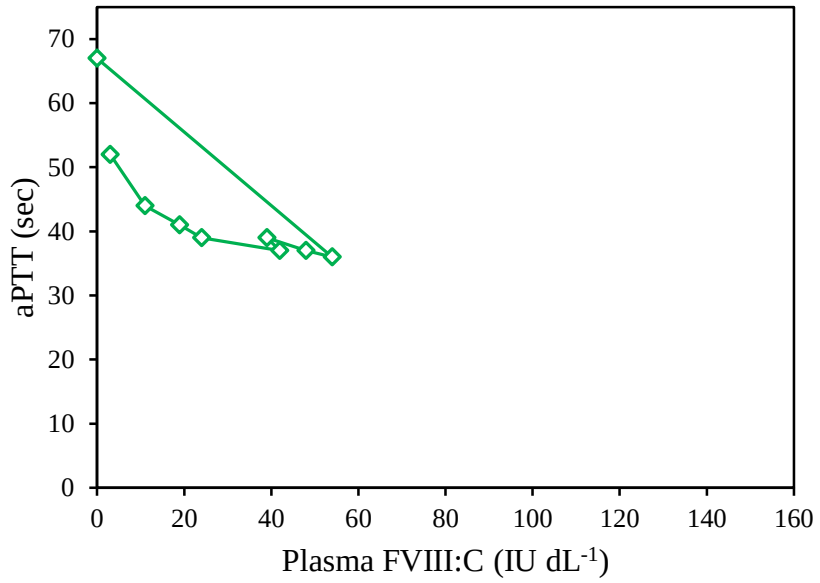
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



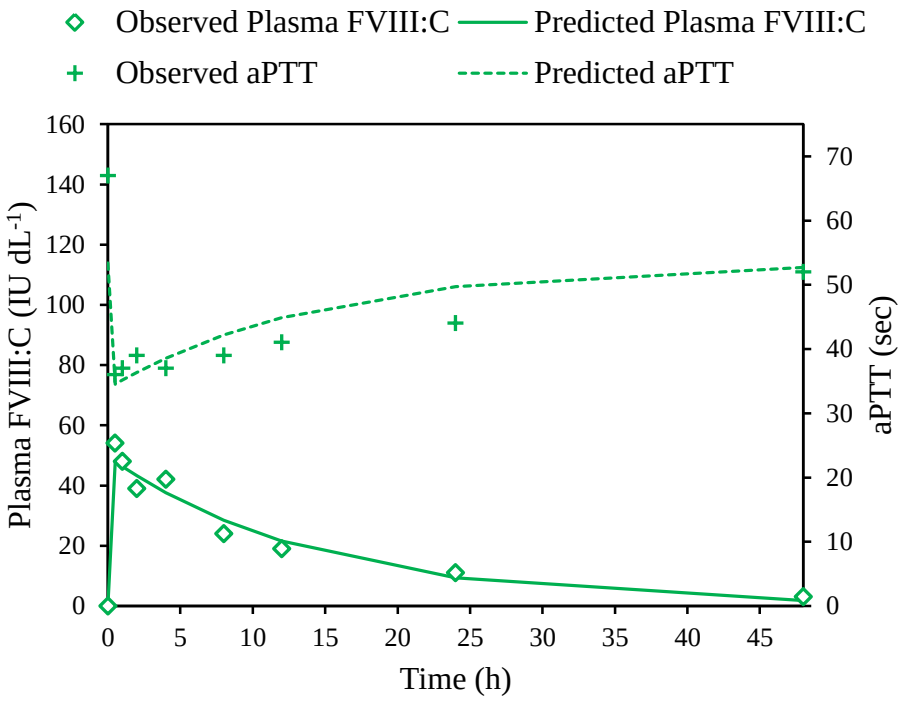
aPTT-Time Profile



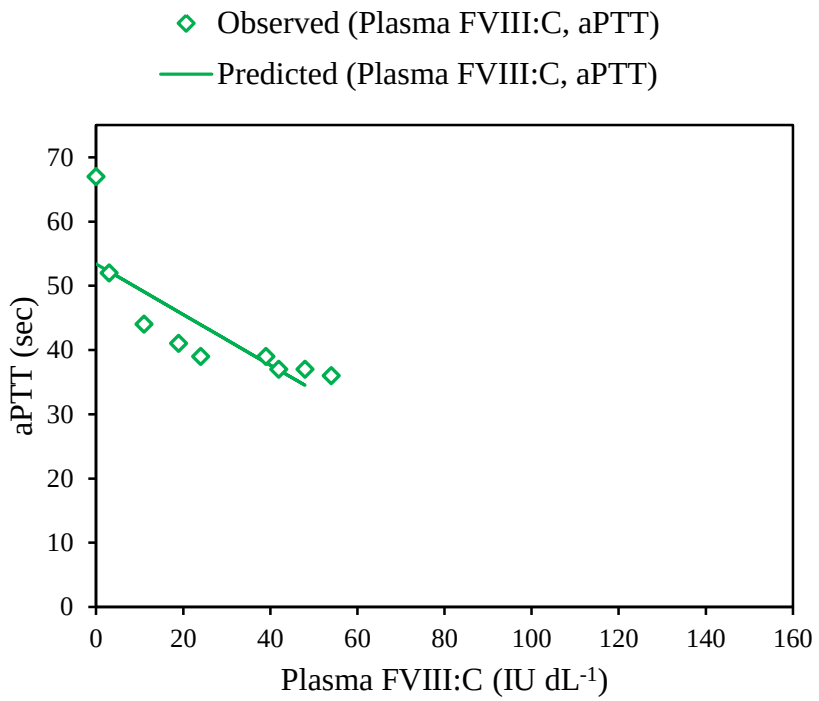
aPTT-Plasma FVIII:C Profile



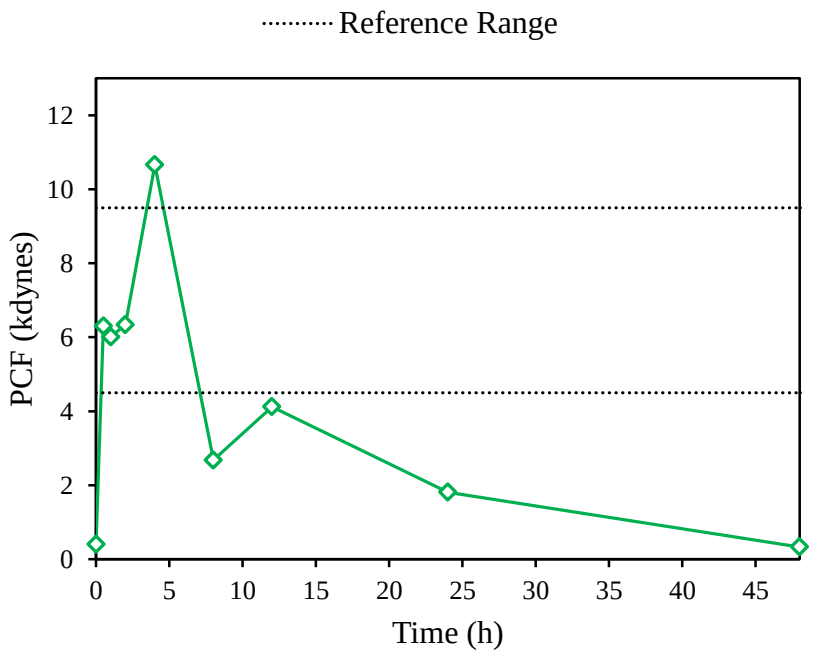
aPTT PK/PD Linear Model Time Dependent Profile



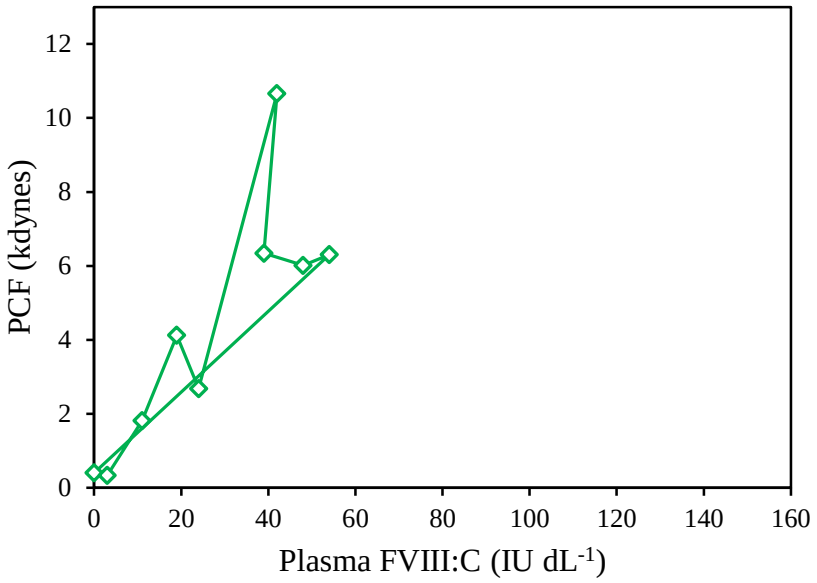
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



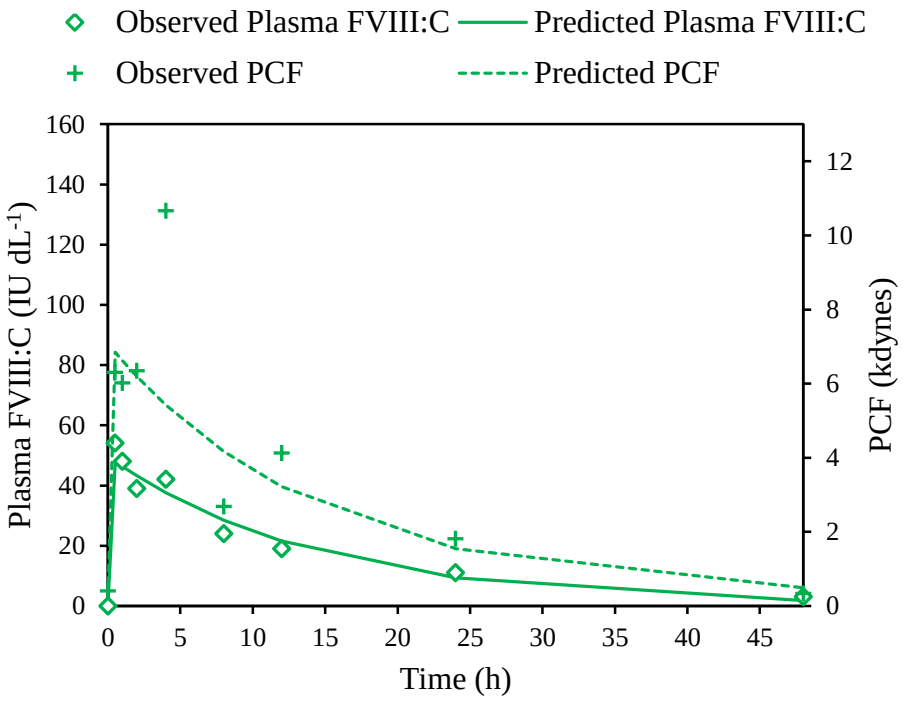
PCF-Time Profile



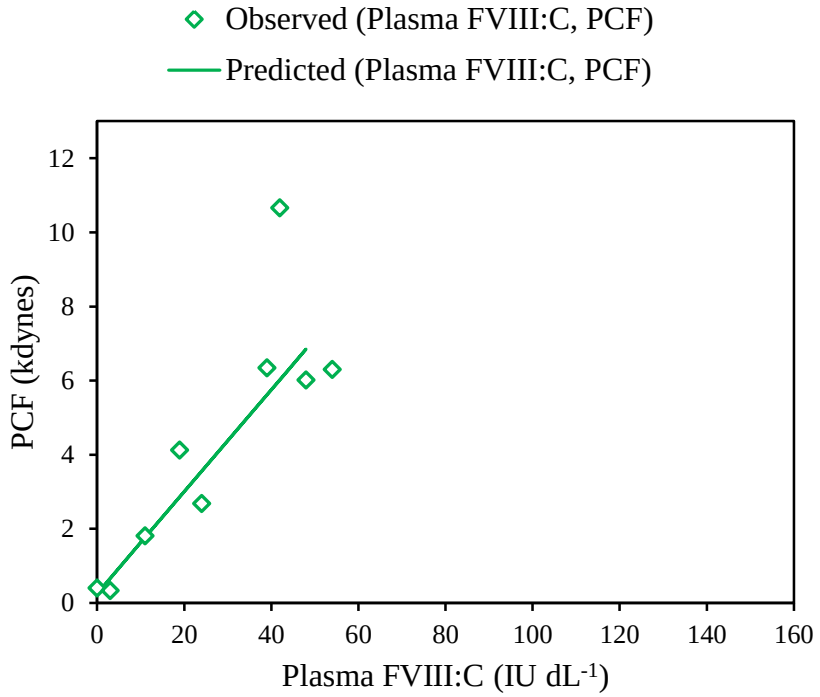
PCF-Plasma FVIII:C Profile



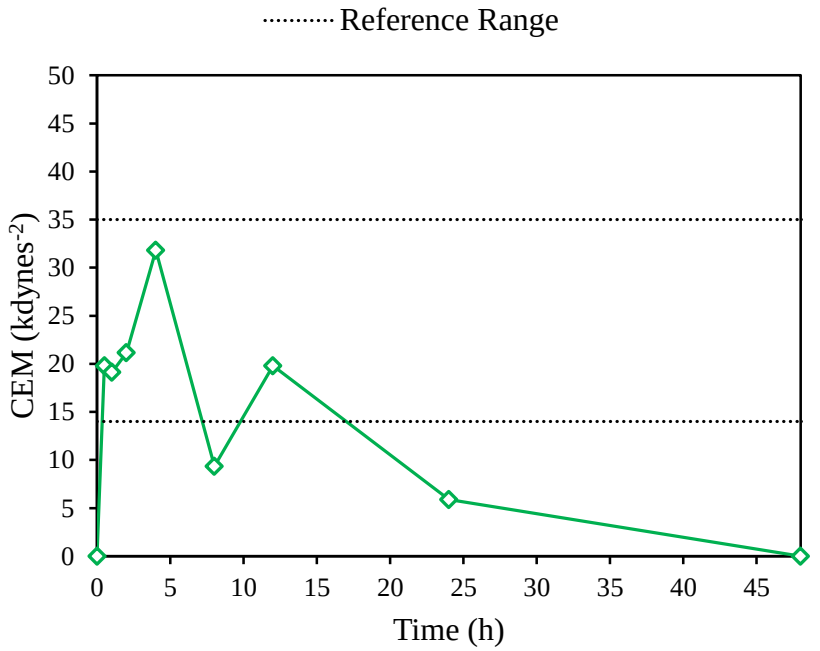
PCF PK/PD Linear Model Time Dependent Profile



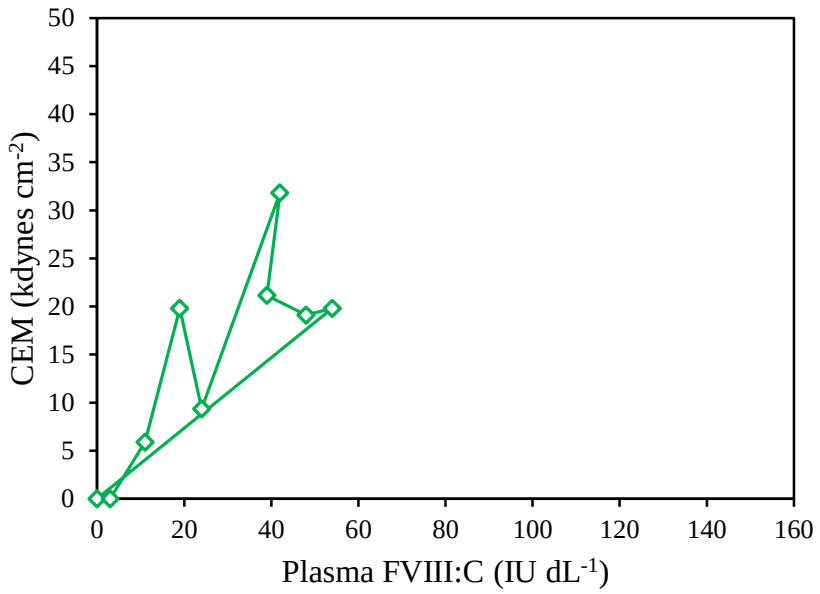
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



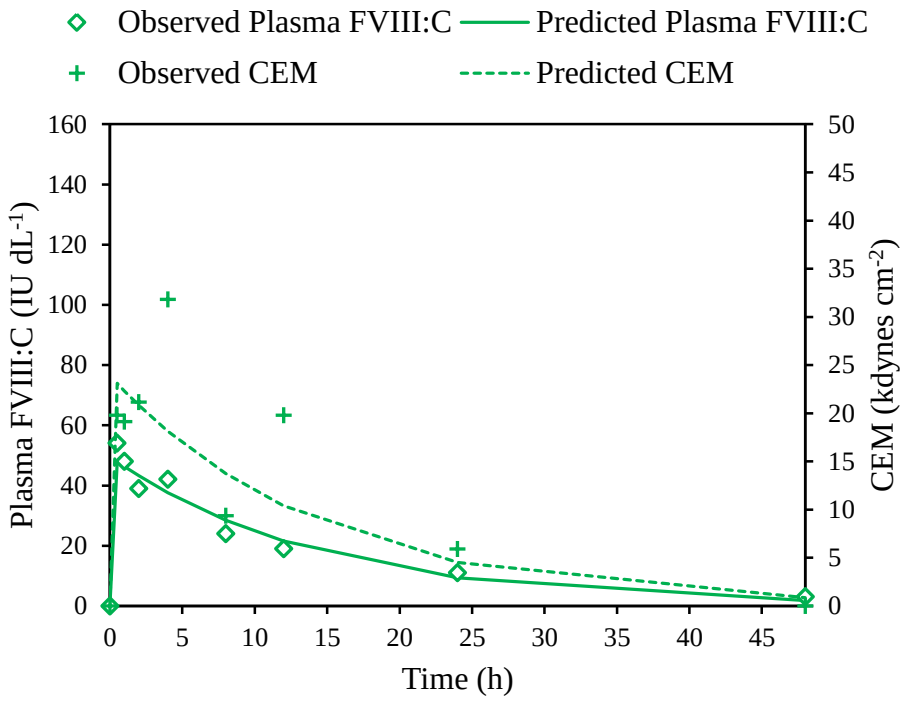
CEM-Time Profile



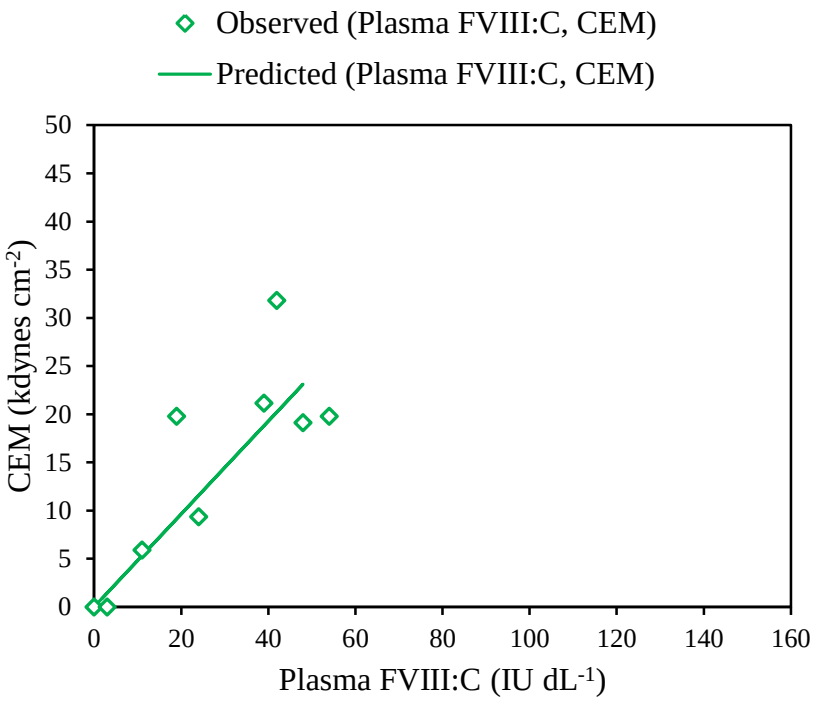
CEM-Plasma FVIII:C Profile



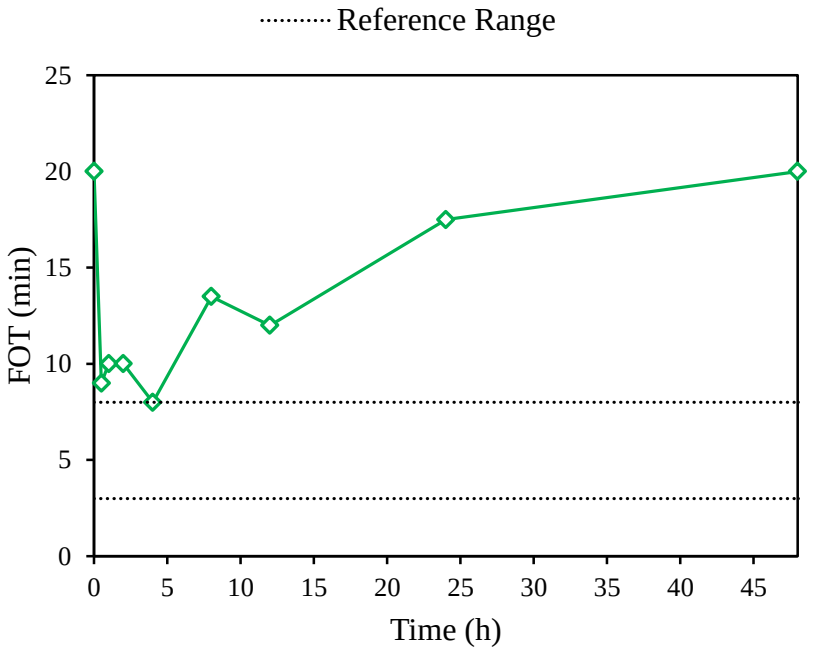
CEM PK/PD Linear Model Time Dependent Profile



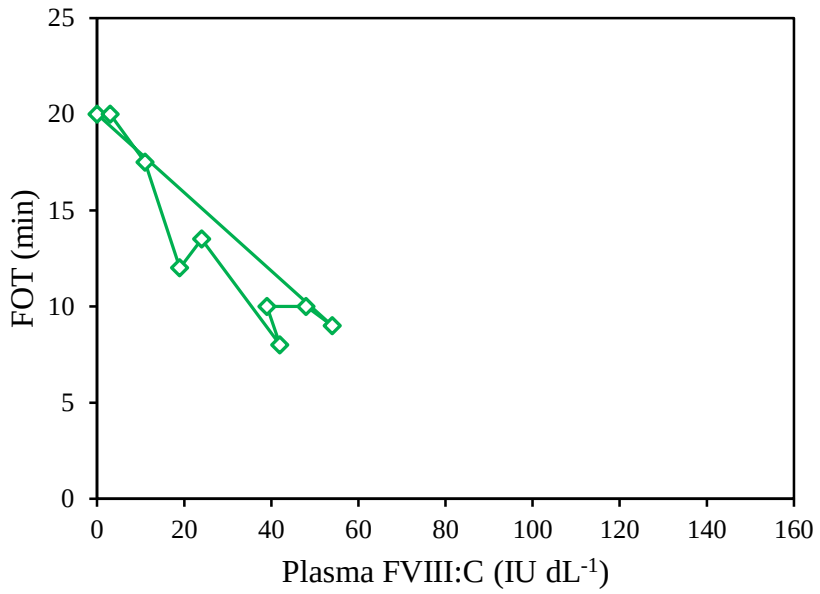
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



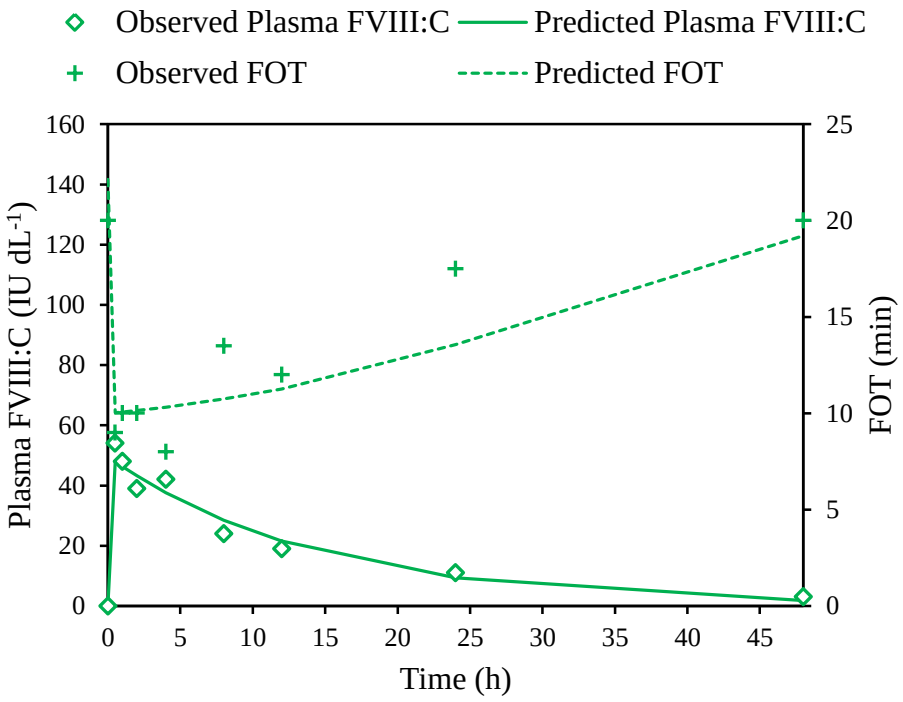
FOT-Time Profile



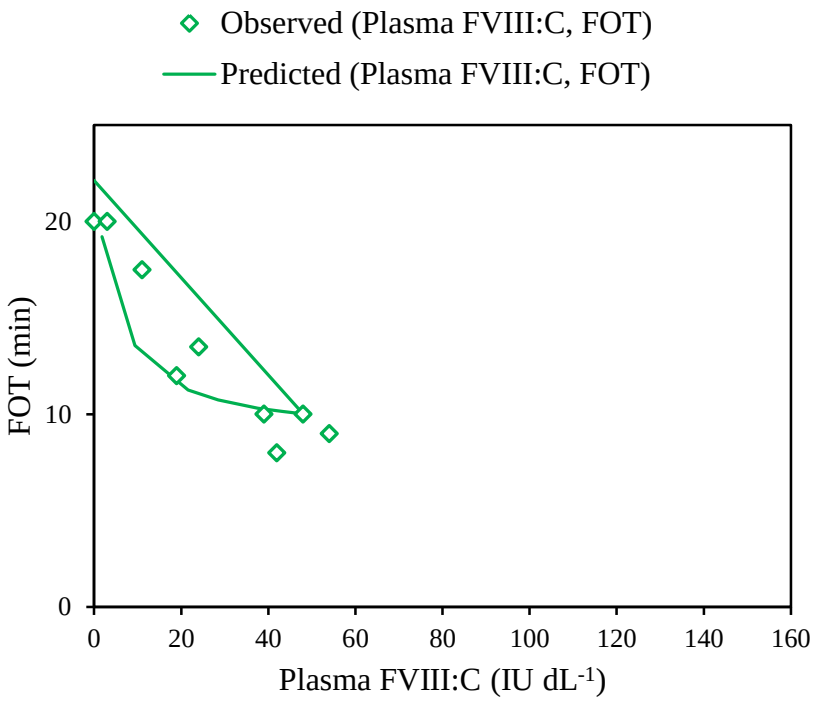
FOT-Plasma FVIII:C Profile



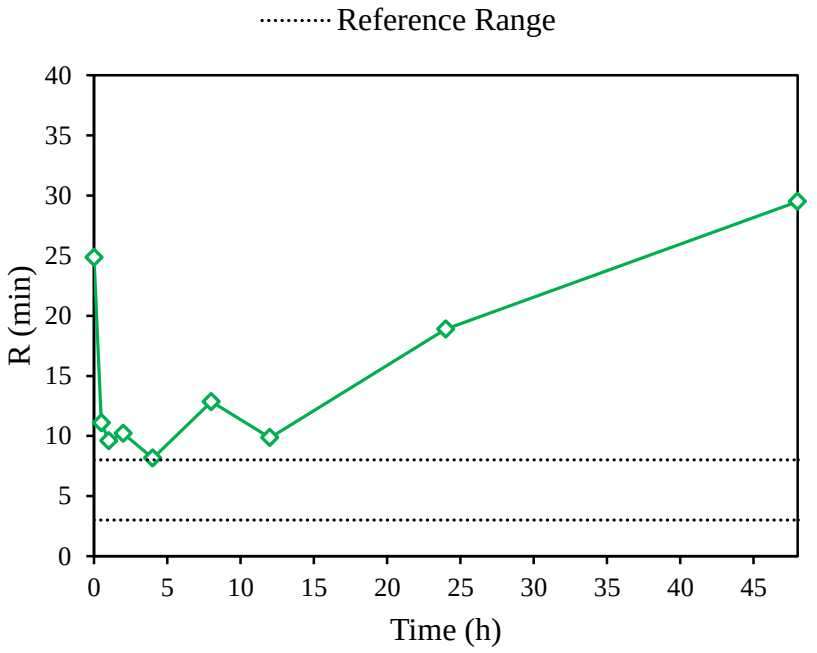
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



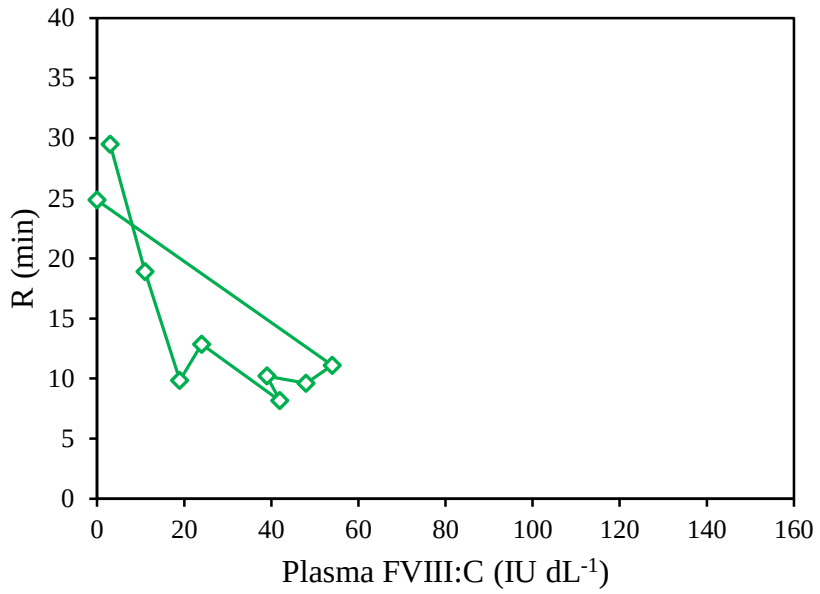
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



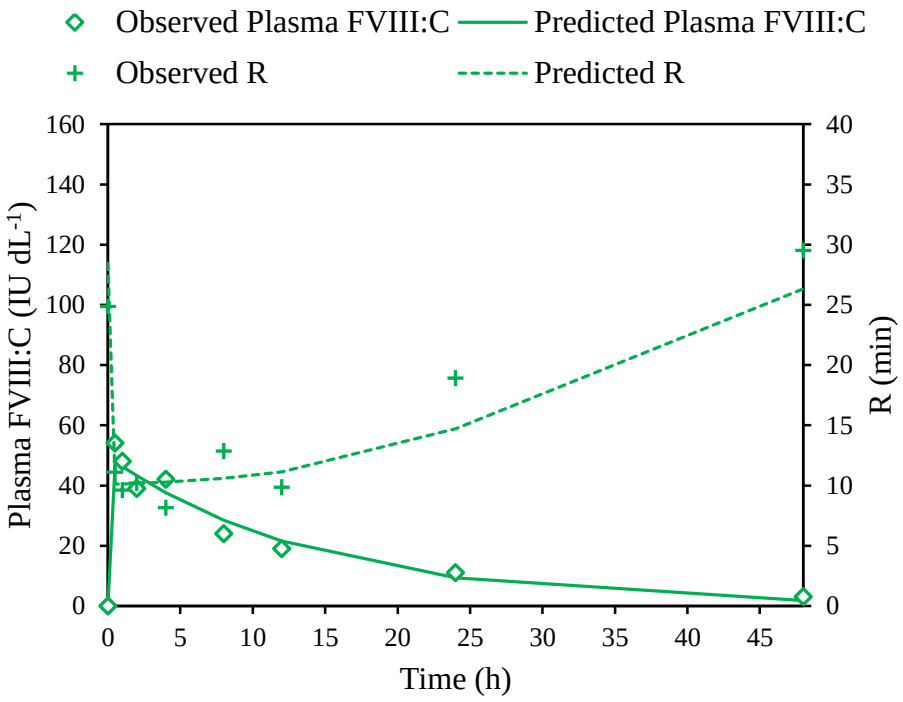
R-Time Profile



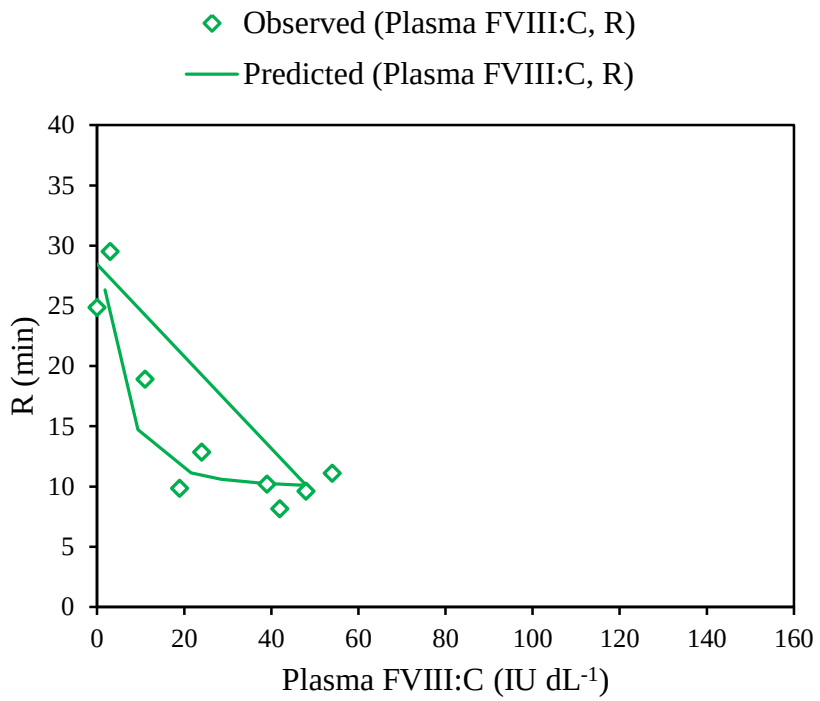
R-Plasma FVIII:C Profile



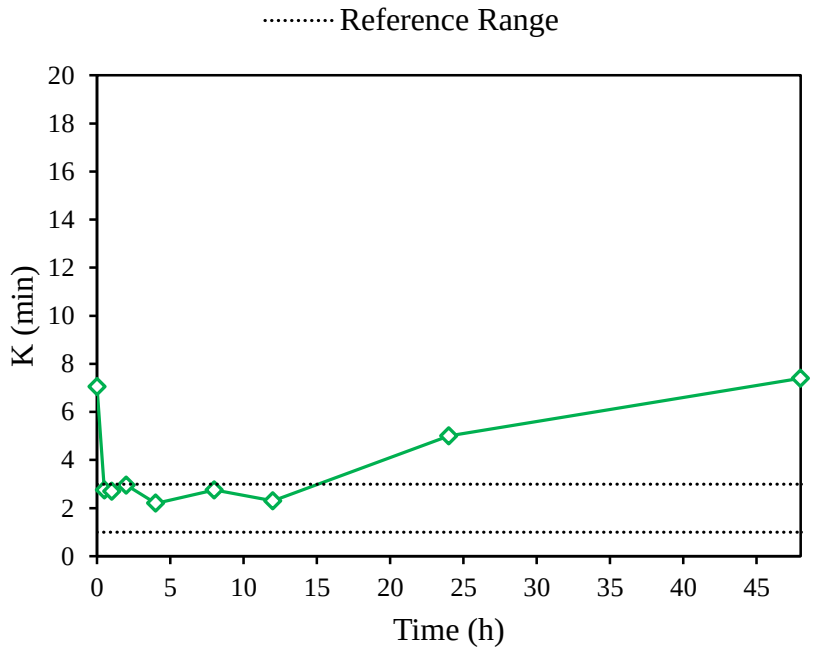
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



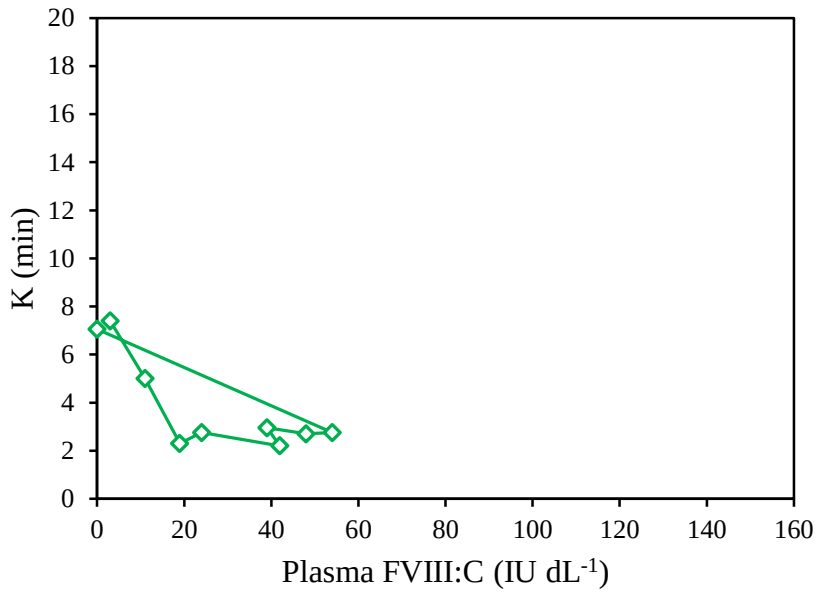
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



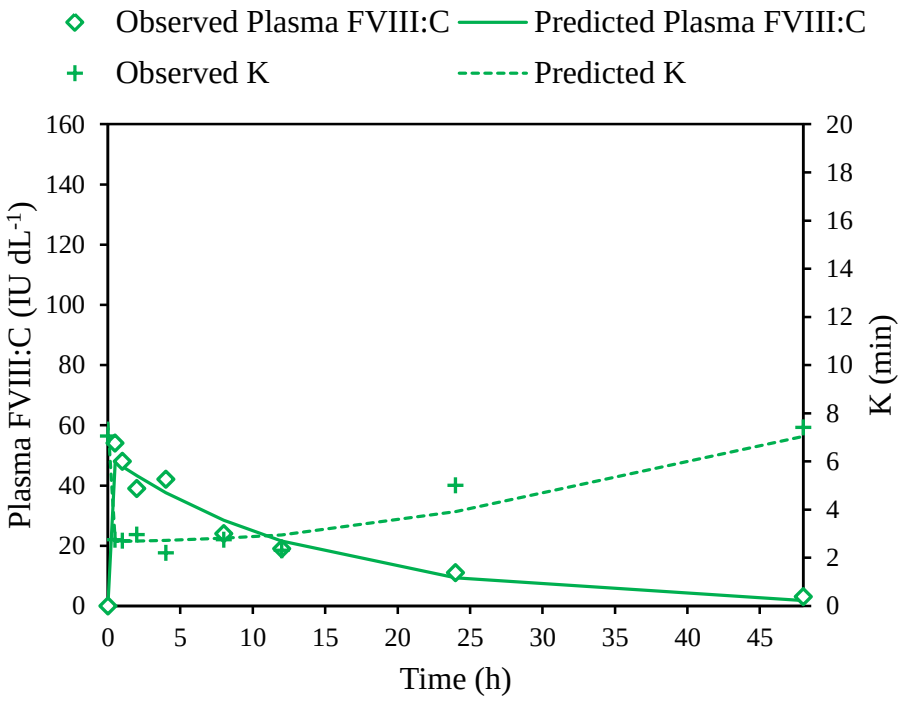
K-Time Profile



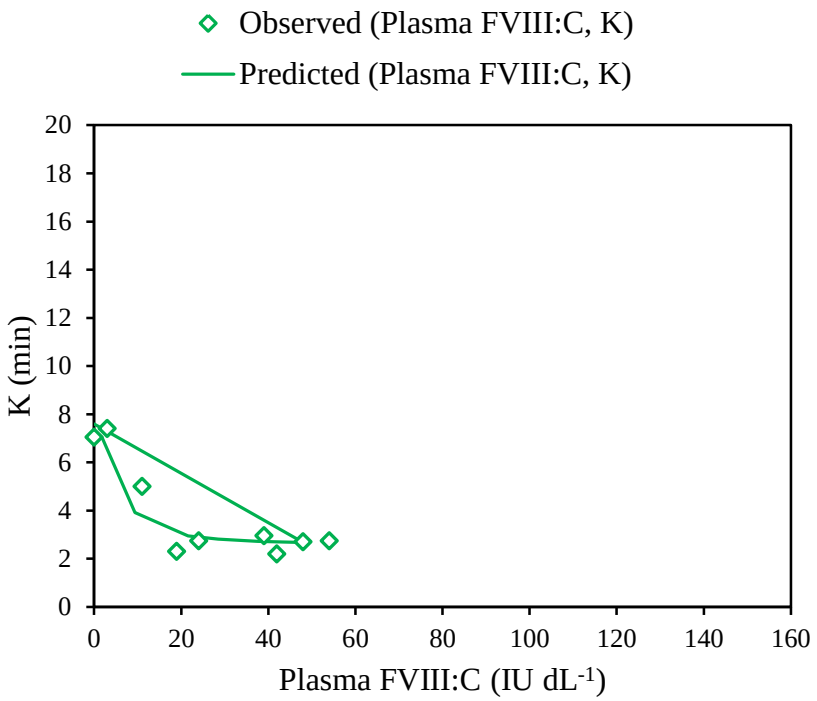
K-Plasma FVIII:C Profile



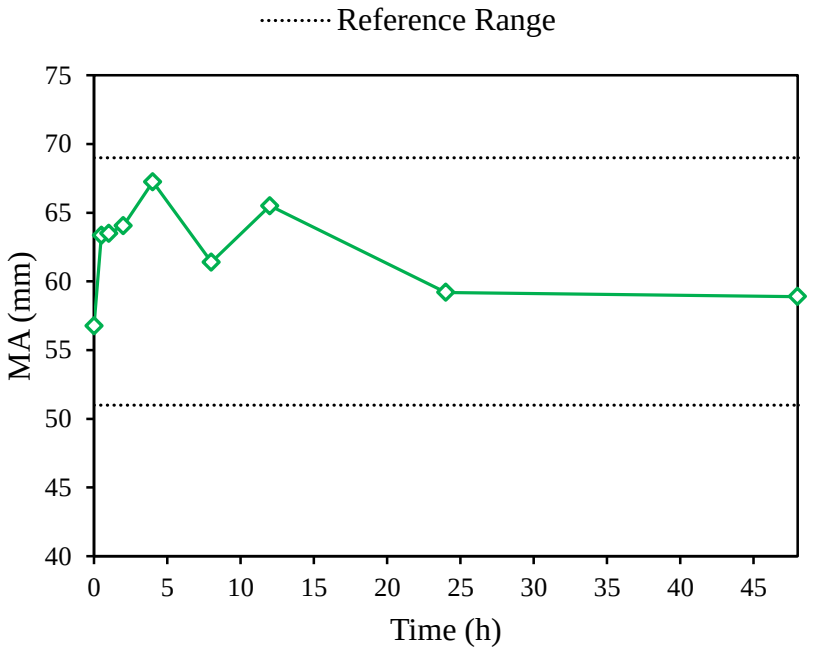
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

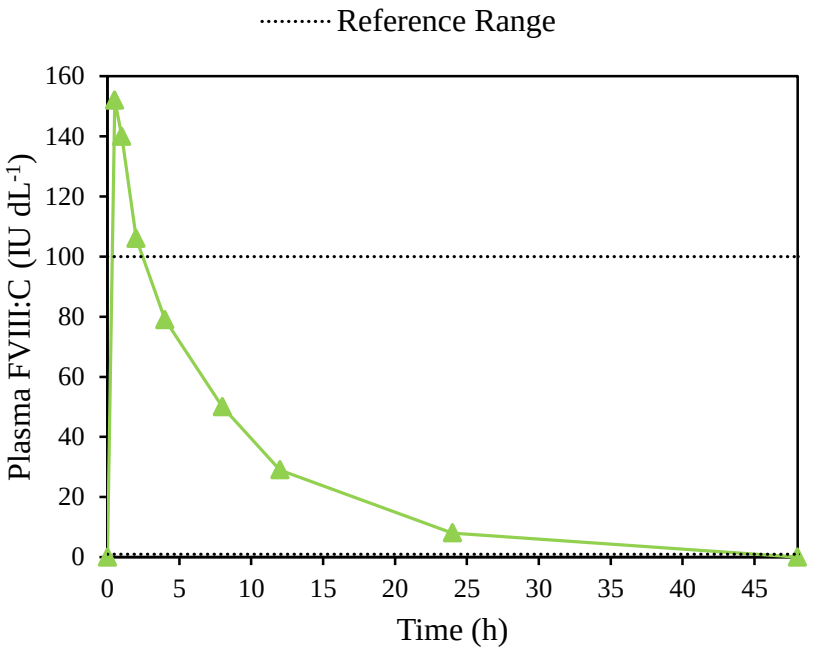


MA-Time Profile

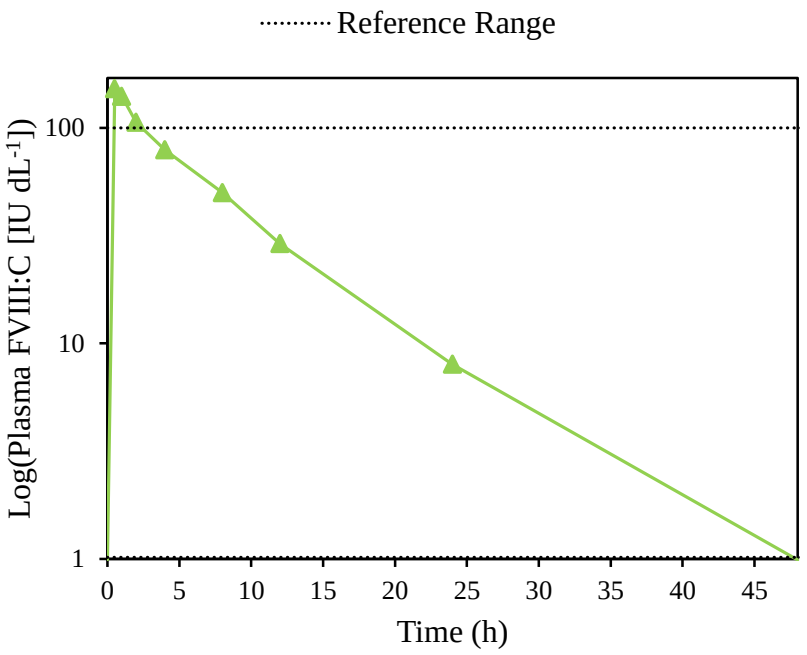


Appendix 11. Individual Profiles for Patient 7

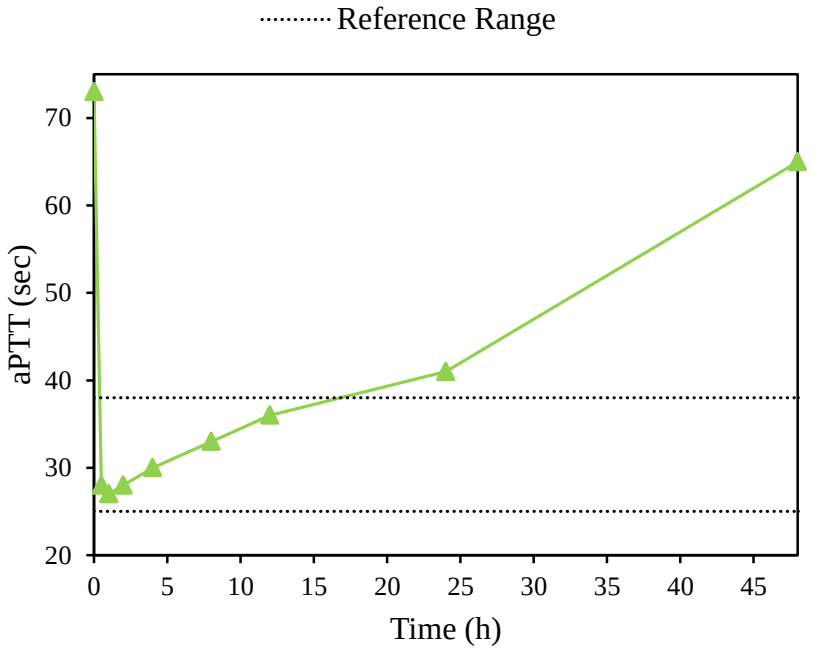
Plasma FVIII:C-Time Profiles



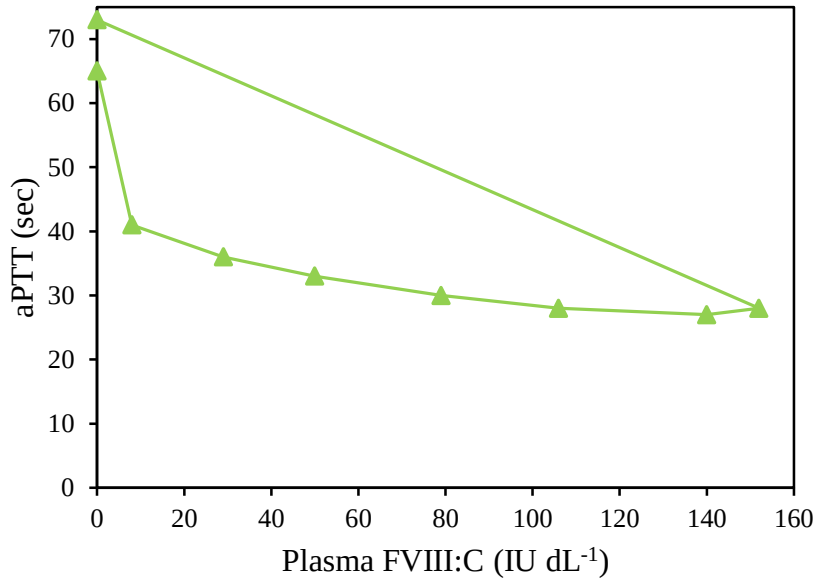
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



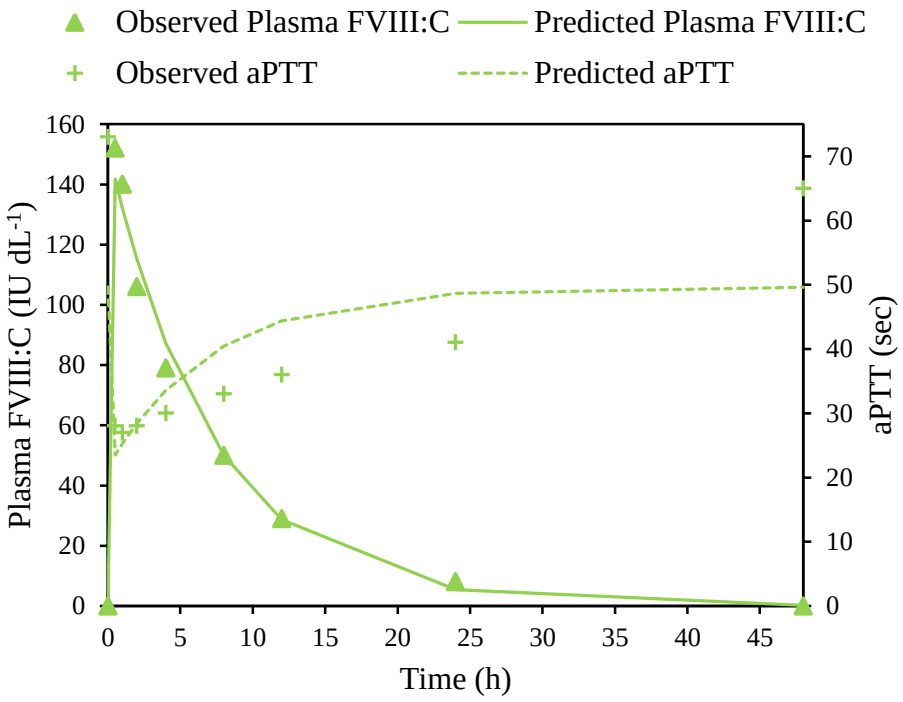
aPTT-Time Profile



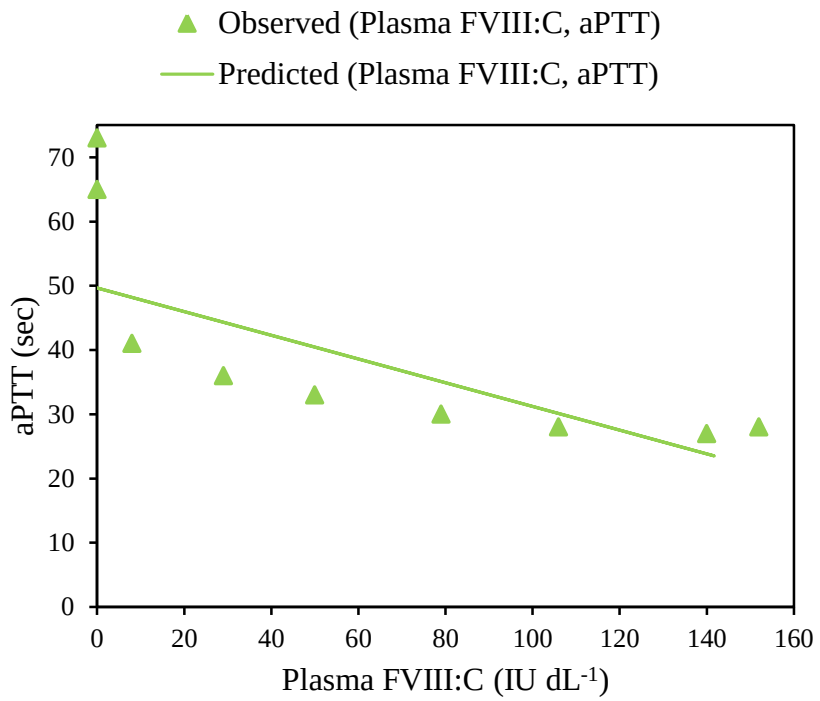
aPTT-Plasma FVIII:C Profile



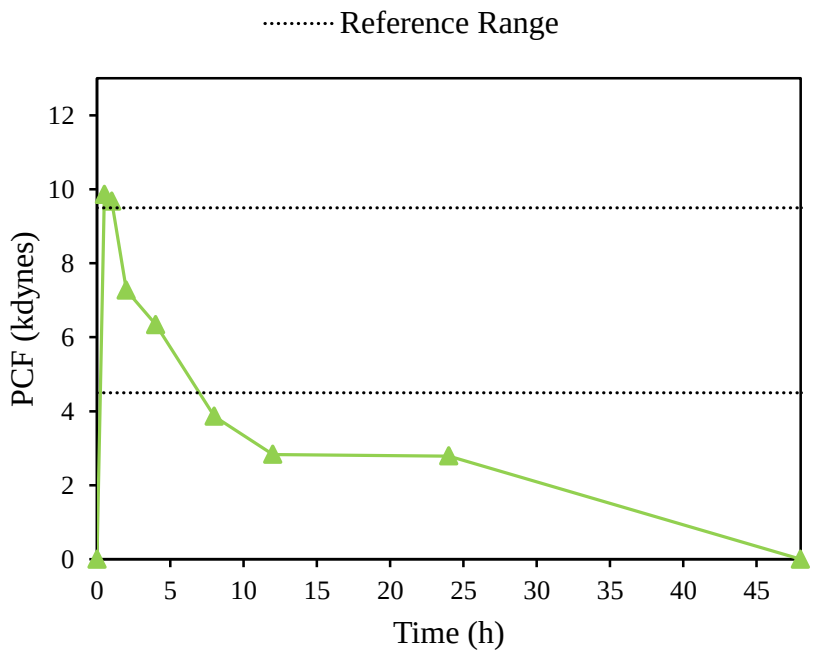
aPTT PK/PD Linear Model Time Dependent Profile



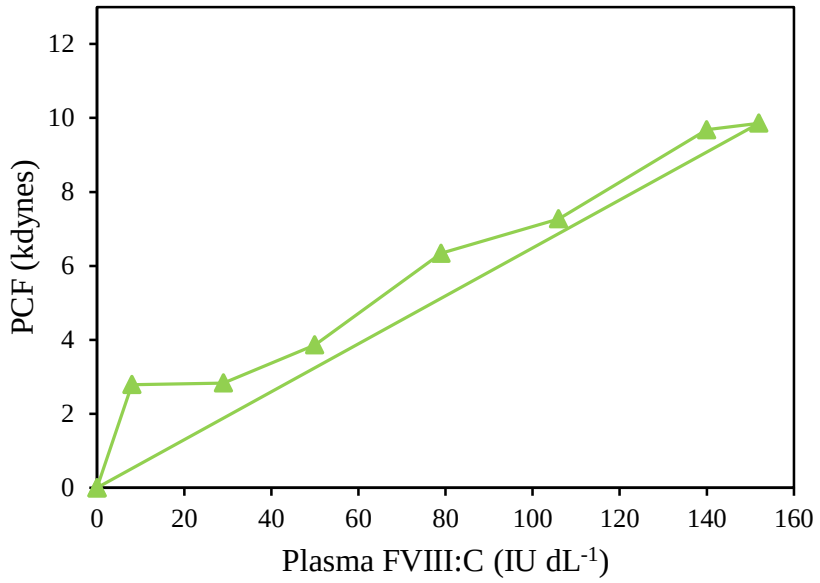
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



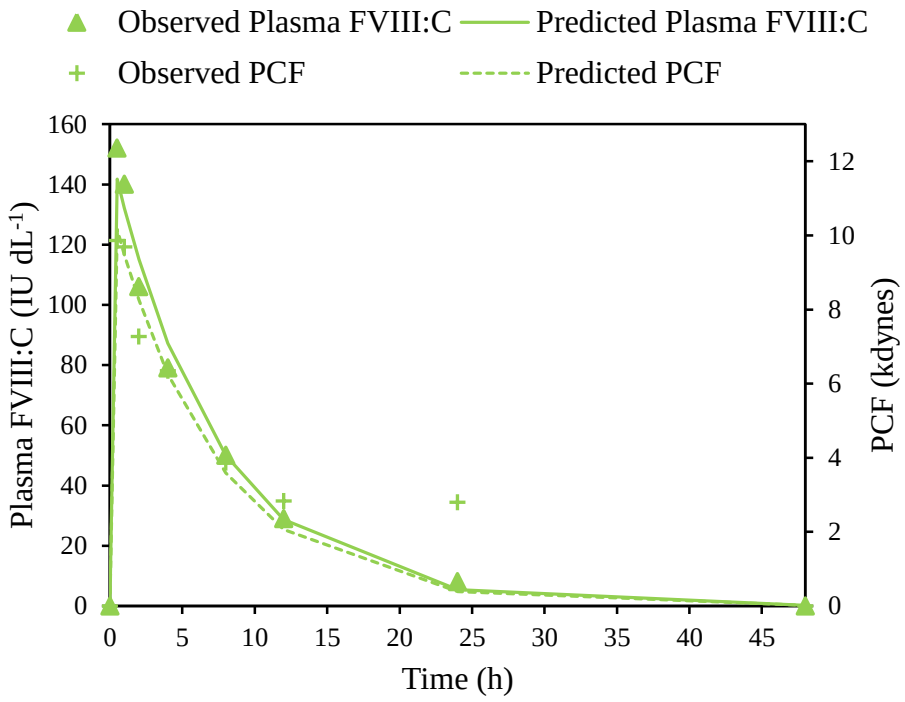
PCF-Time Profile



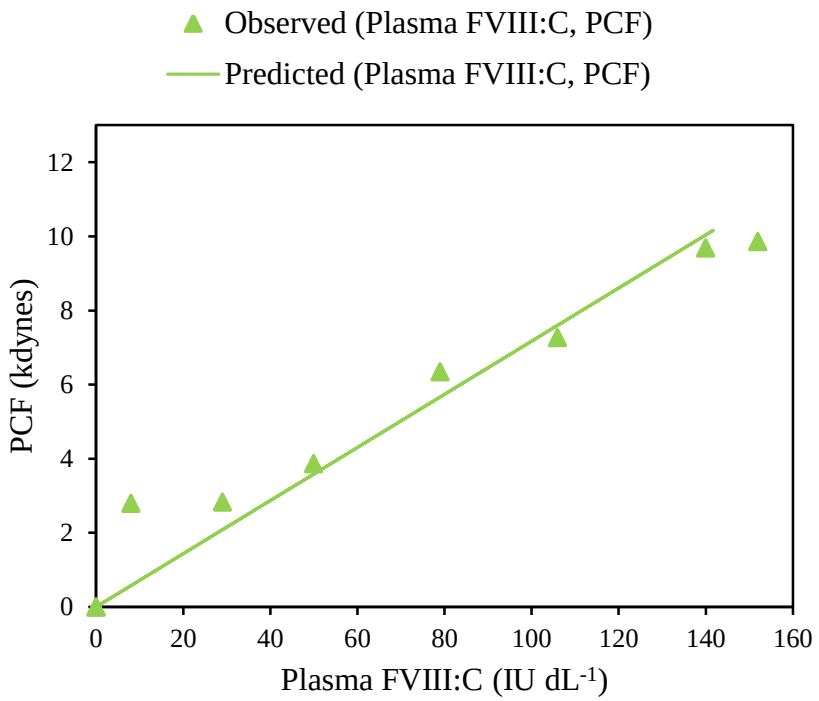
PCF-Plasma FVIII:C Profile



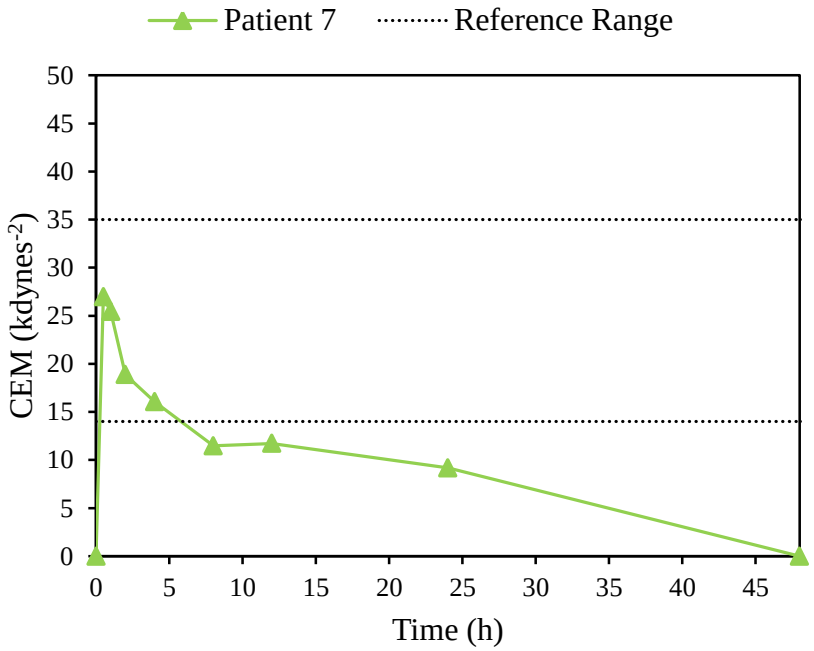
PCF PK/PD Linear Model Time Dependent Profile



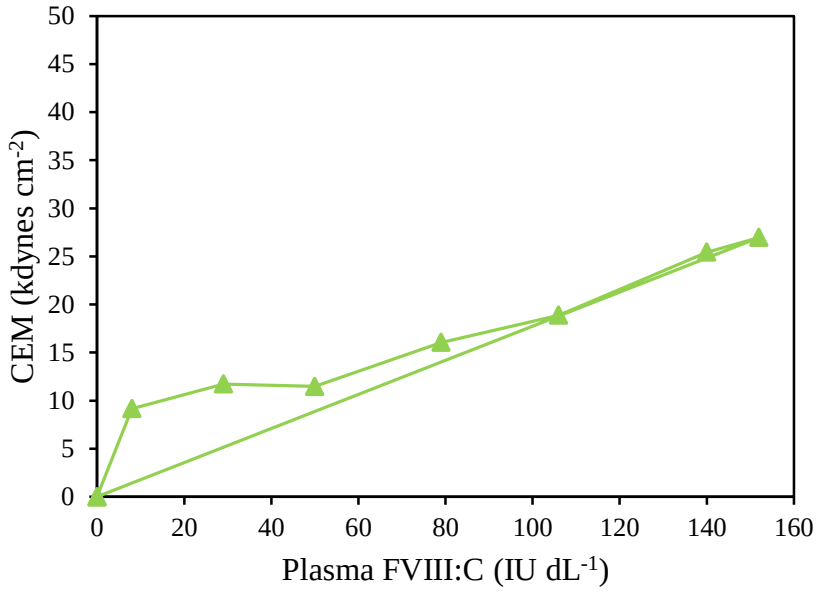
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



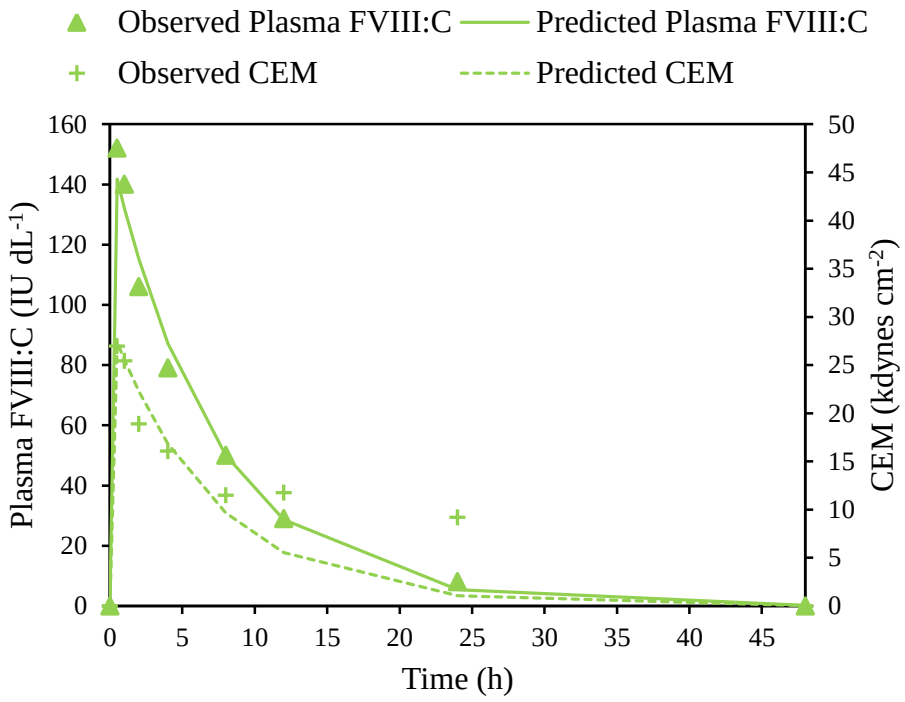
CEM-Time Profile



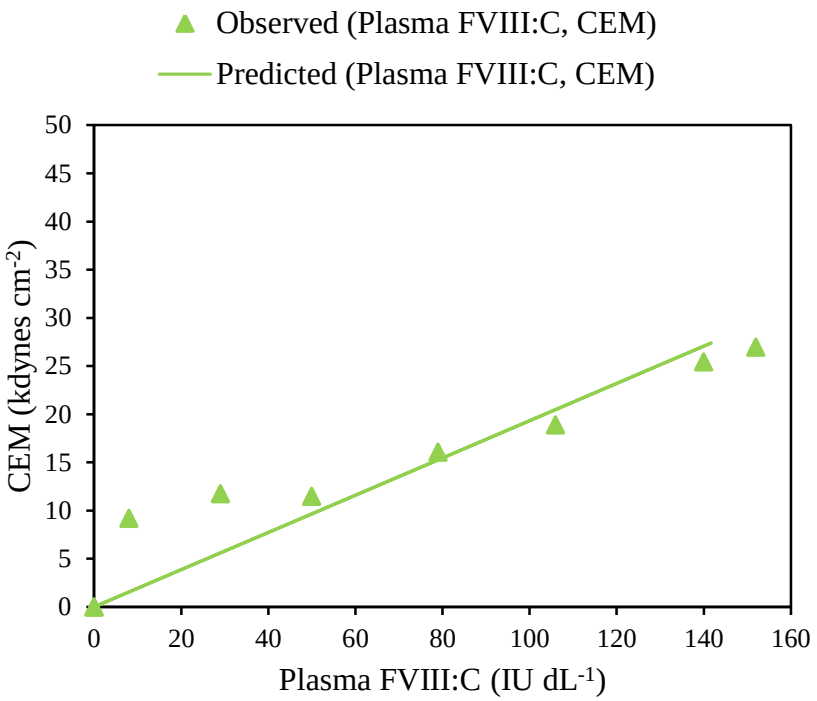
CEM-Plasma FVIII:C Profile



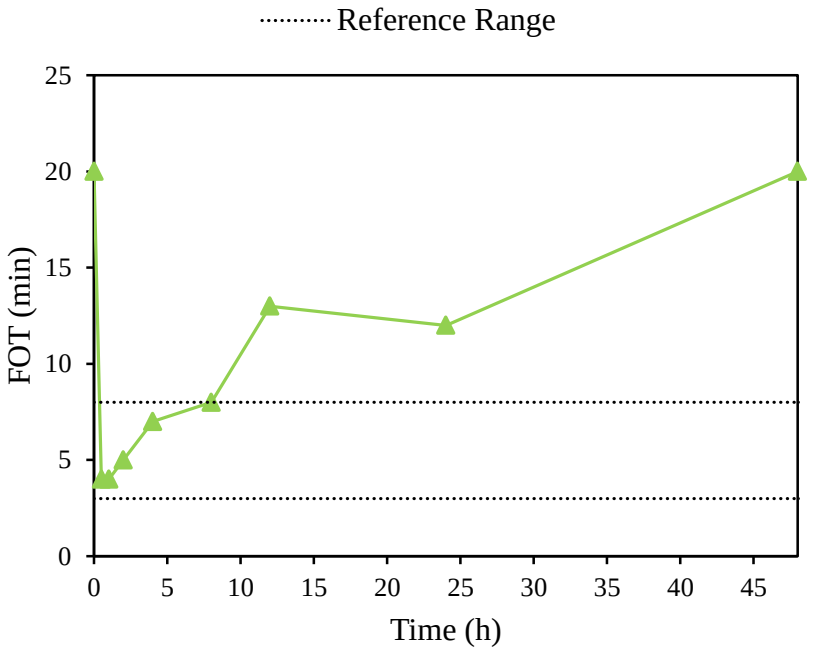
CEM PK/PD Linear Model Time Dependent Profile



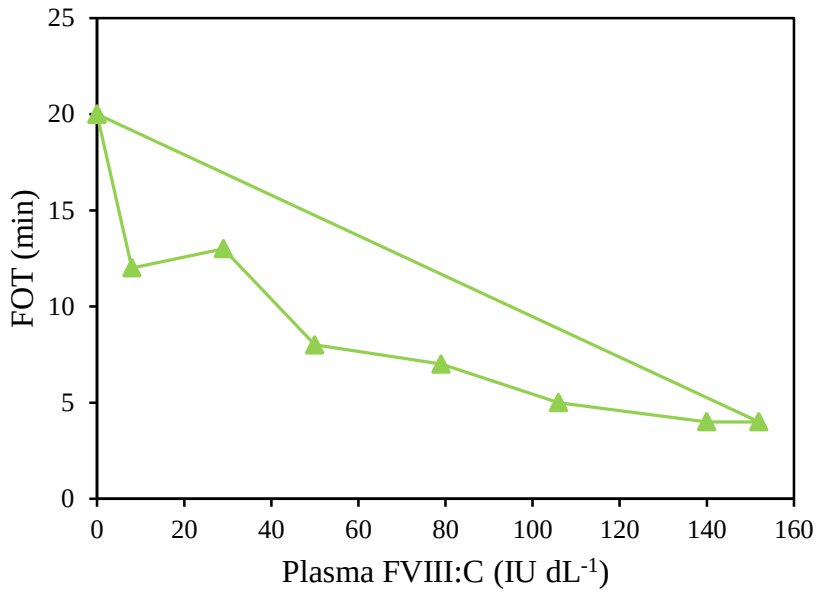
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



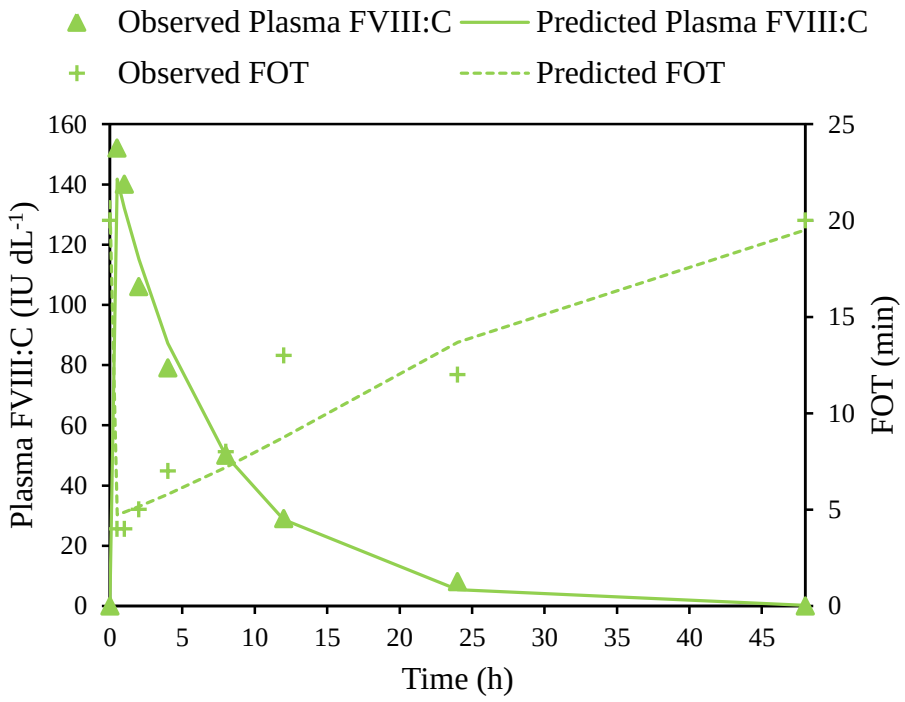
FOT-Time Profile



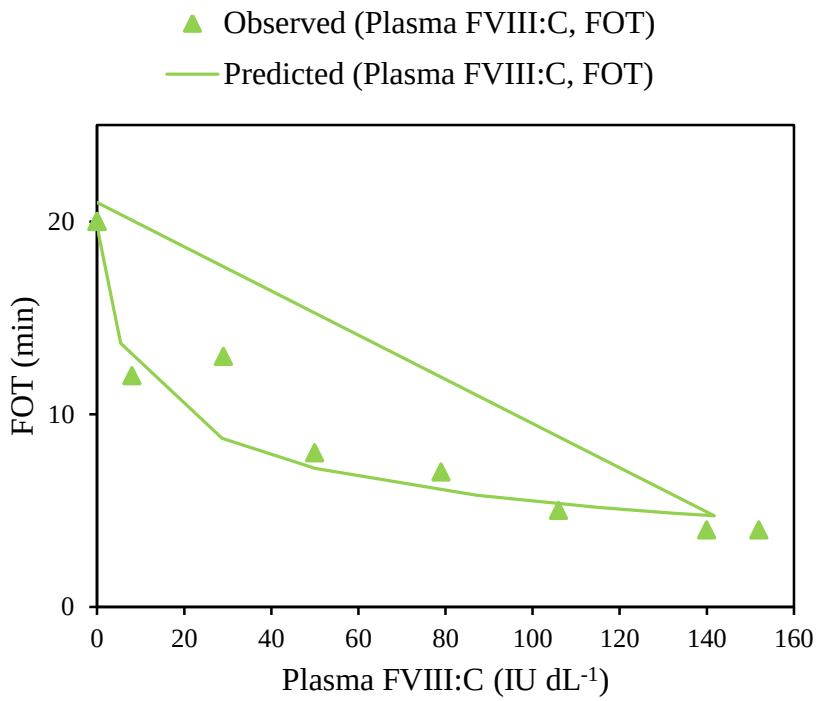
FOT-Plasma FVIII:C Profile



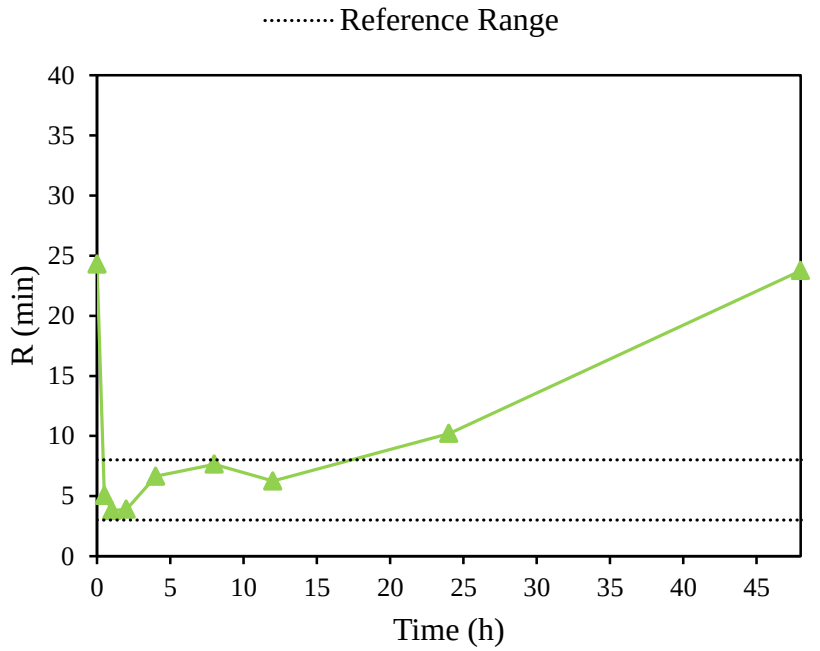
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



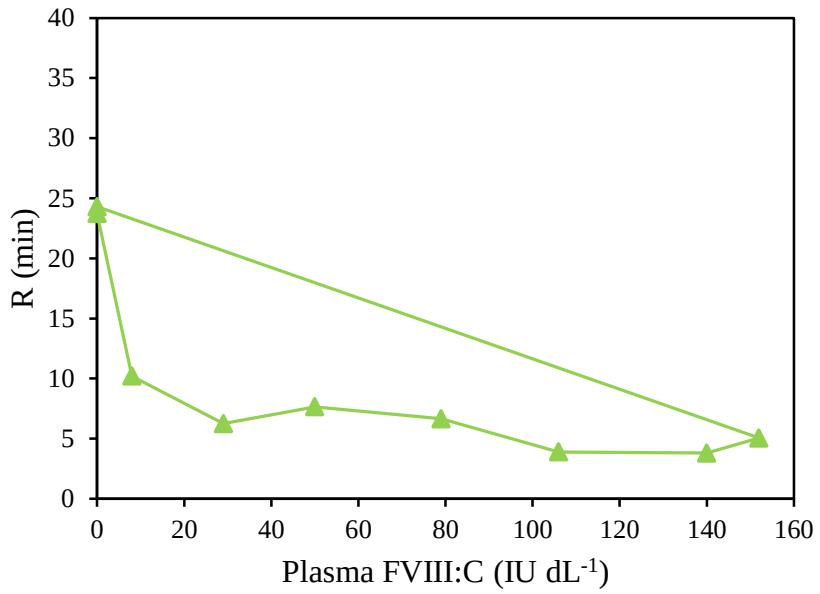
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



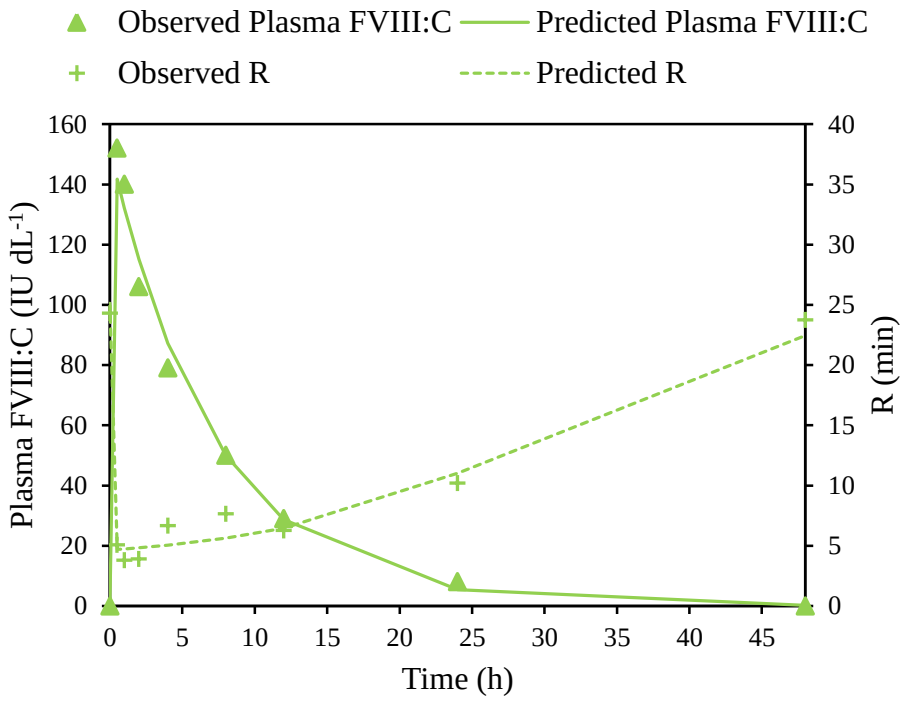
R-Time Profile



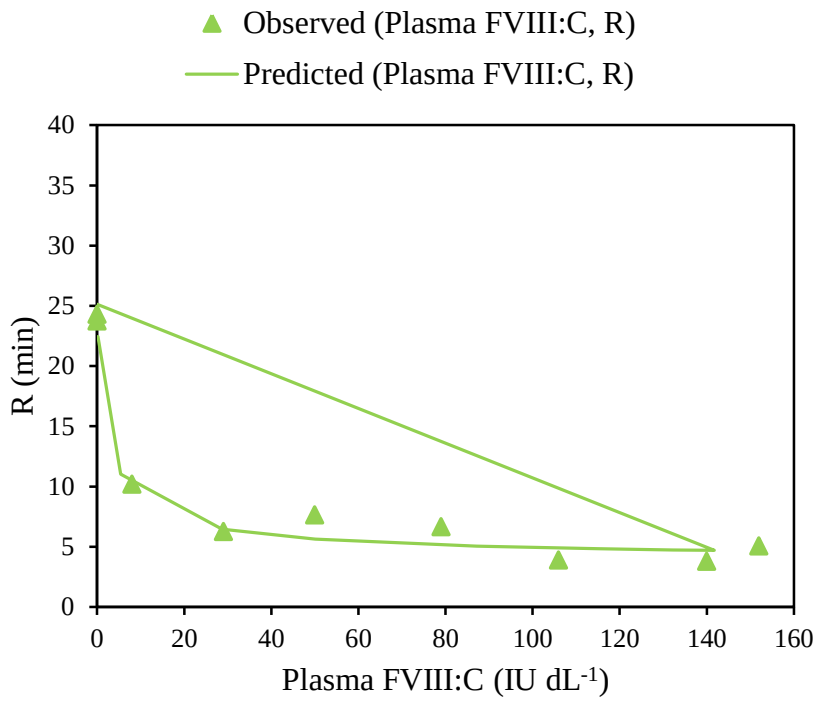
R-Plasma FVIII:C Profile



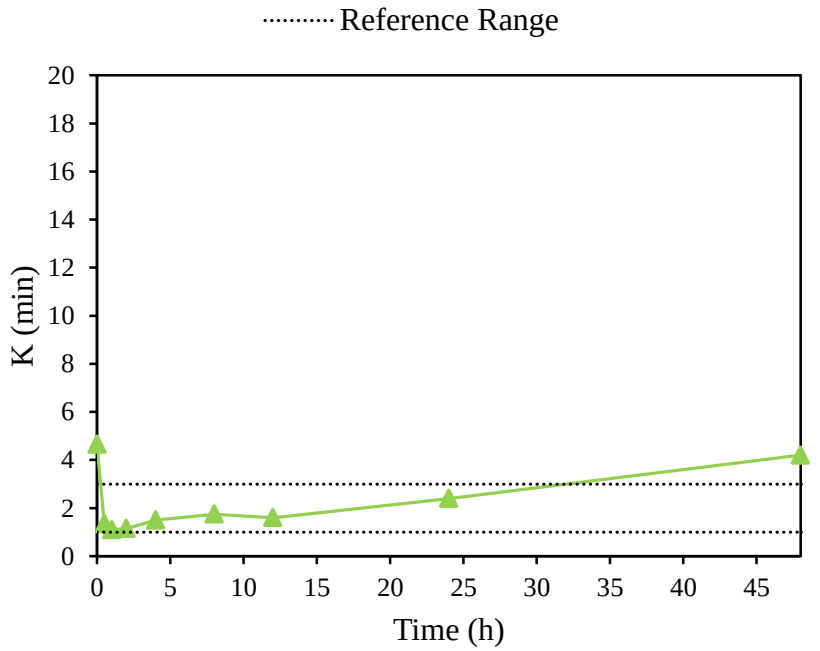
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



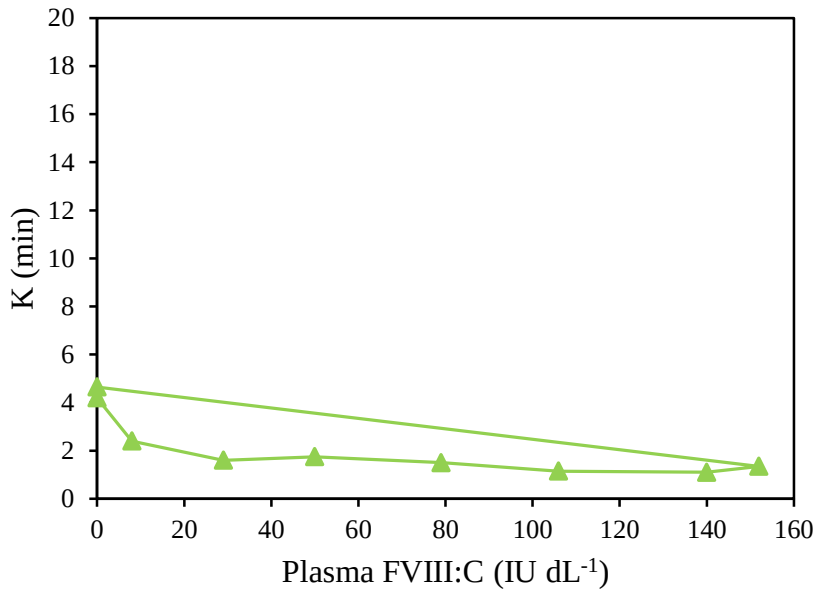
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



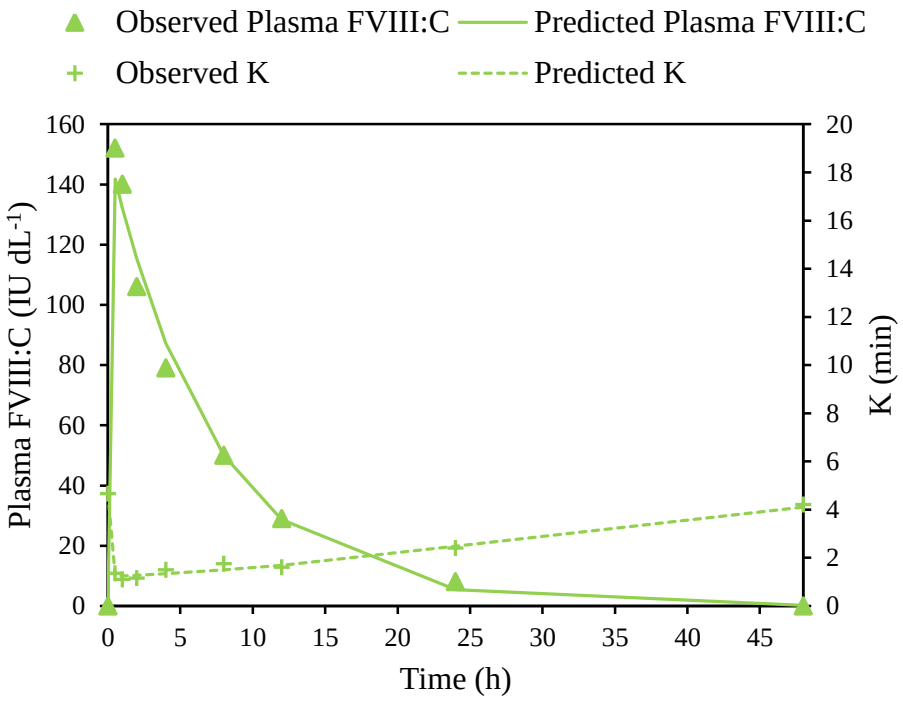
K-Time Profile



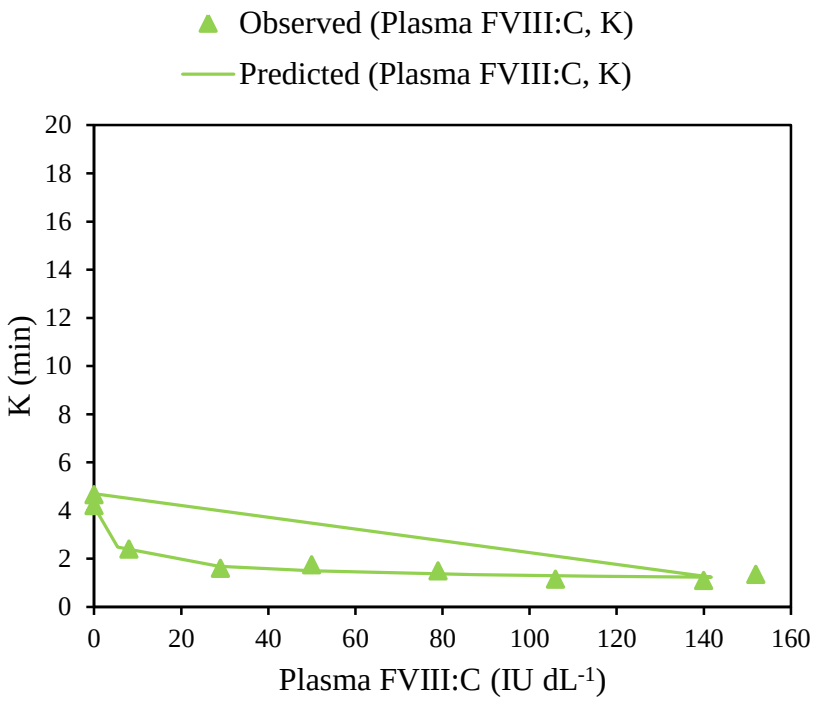
K-Plasma FVIII:C Profile



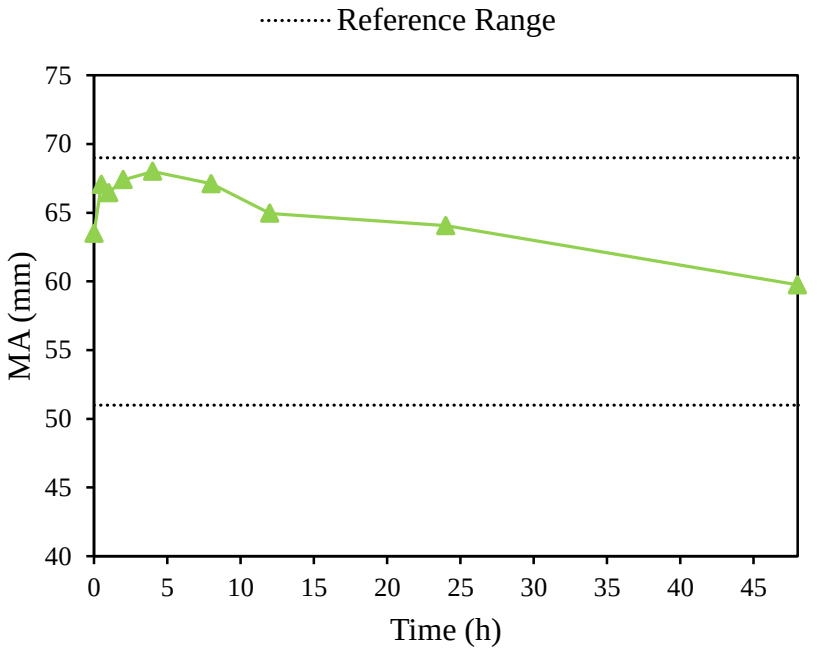
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

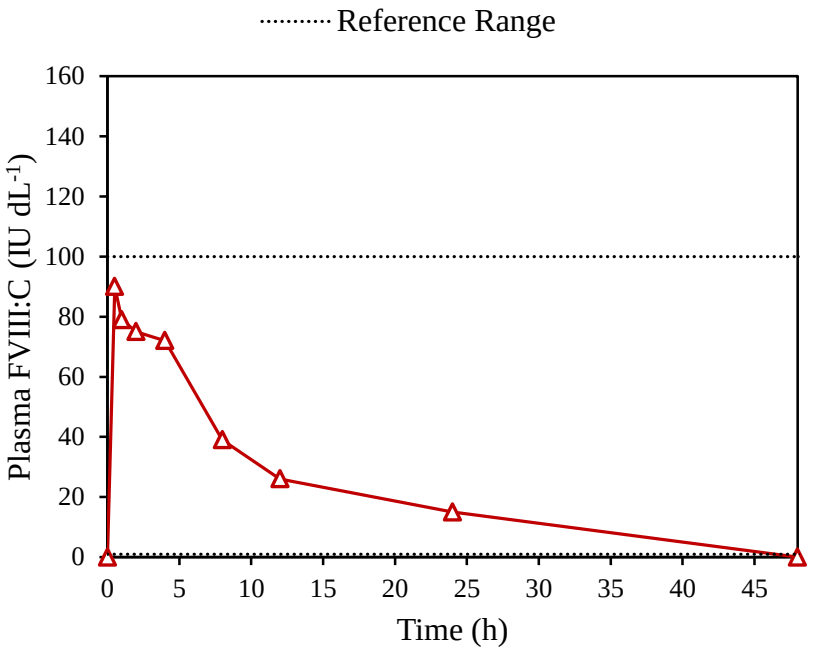


MA-Time Profile

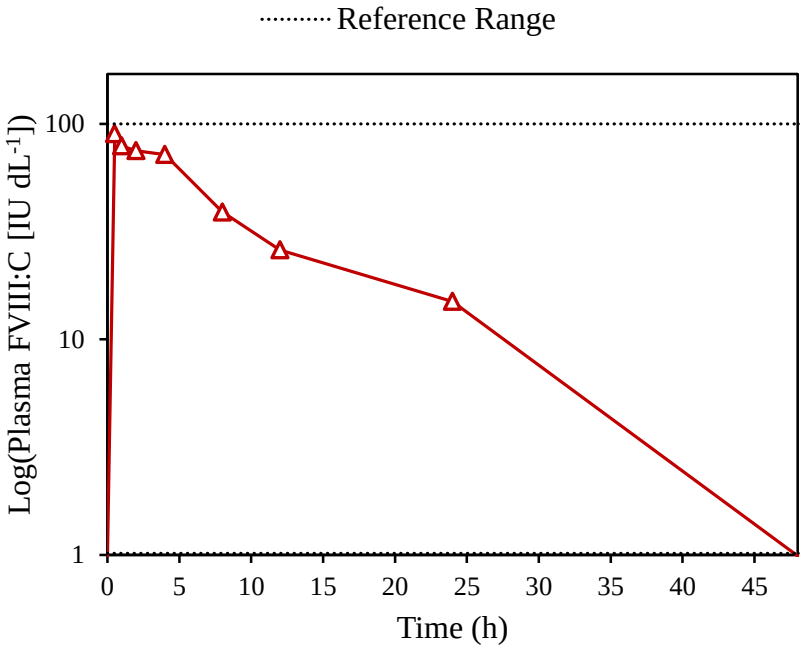


Appendix 12. Individual Profiles for Patient 8

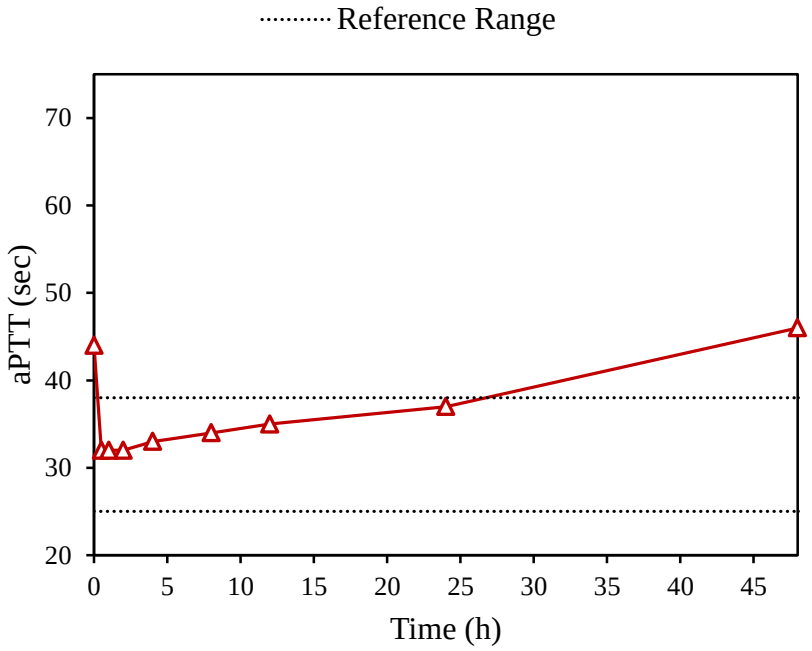
Plasma FVIII:C-Time Profiles



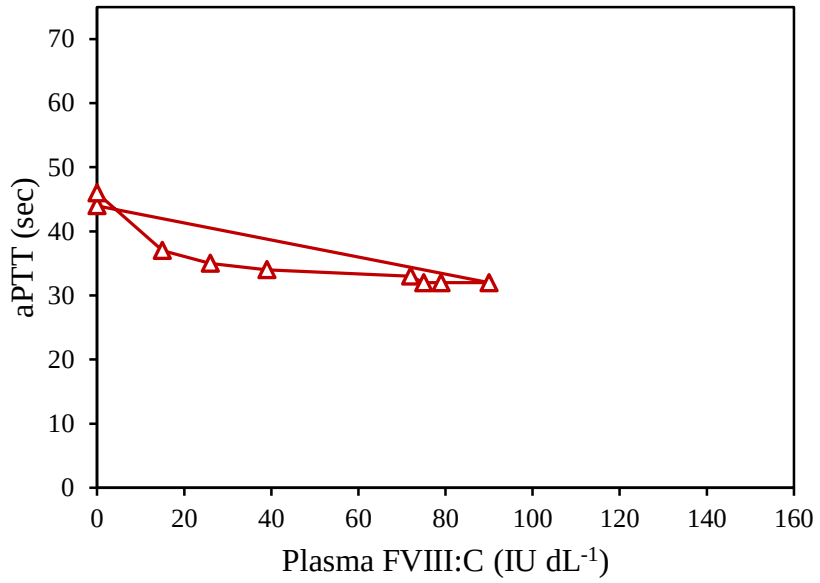
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



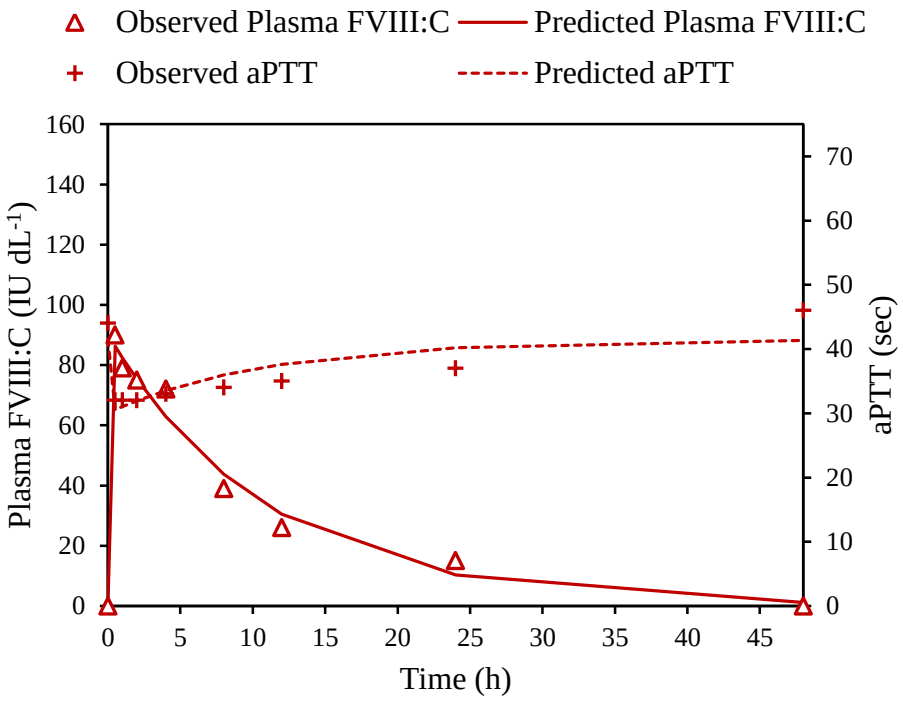
aPTT-Time Profile



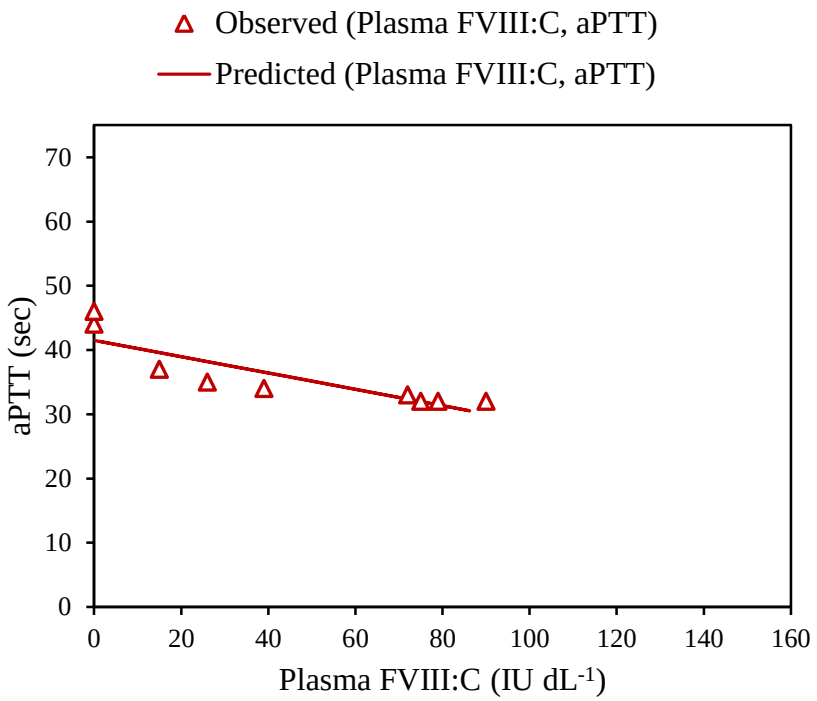
aPTT-Plasma FVIII:C Profile



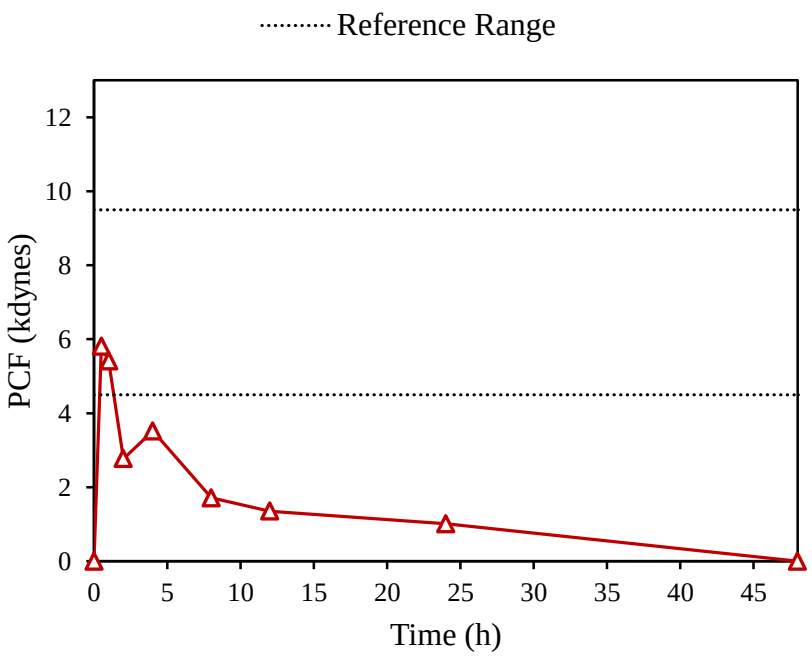
aPTT PK/PD Linear Model Time Dependent Profile



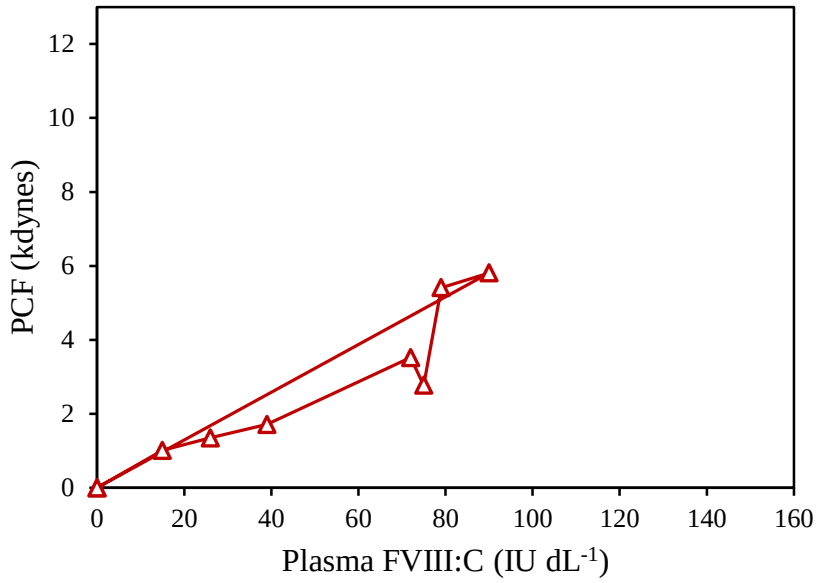
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



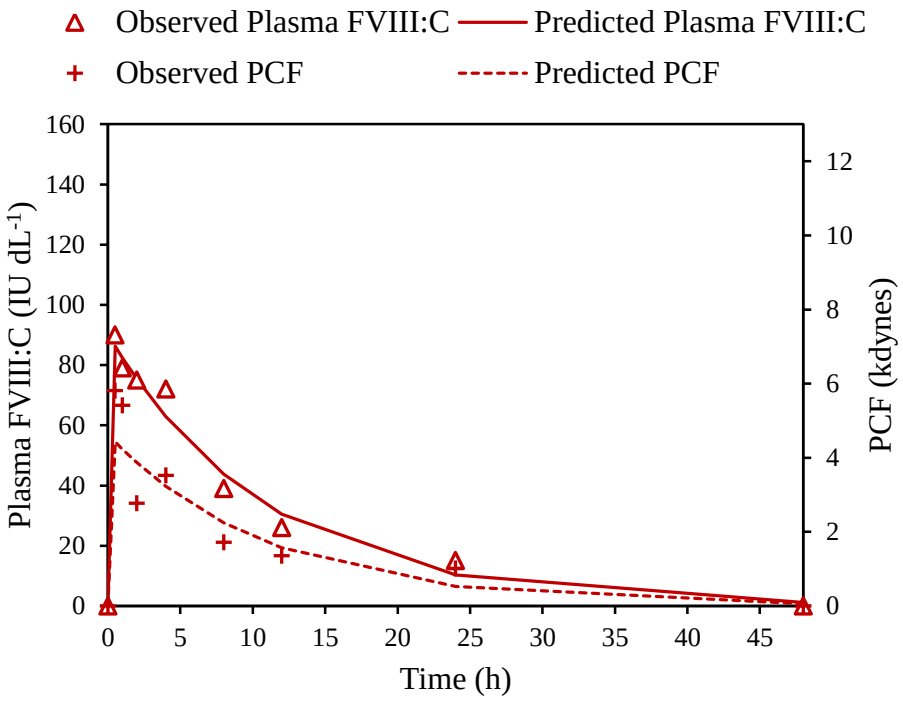
PCF-Time Profile



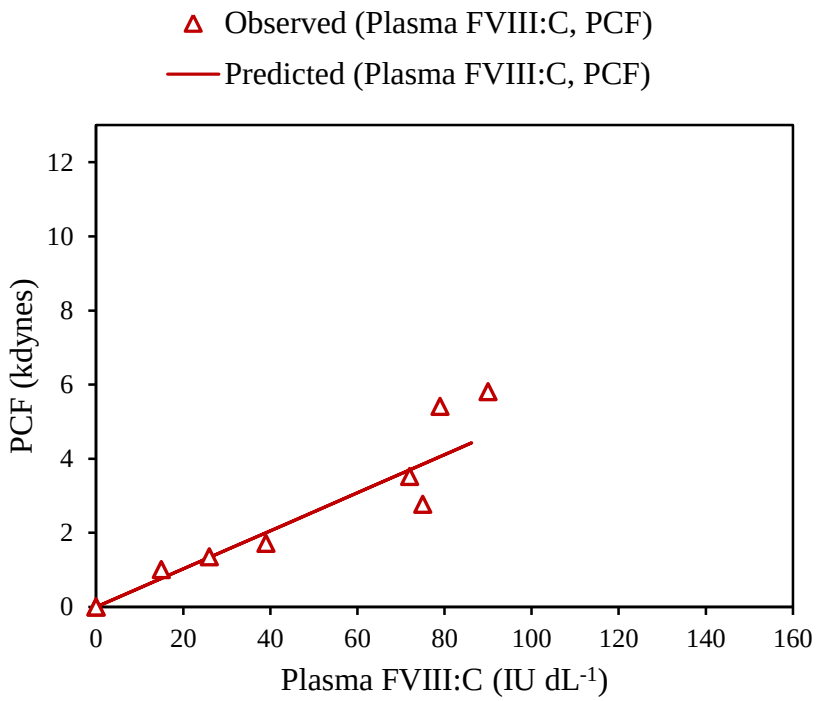
PCF-Plasma FVIII:C Profile



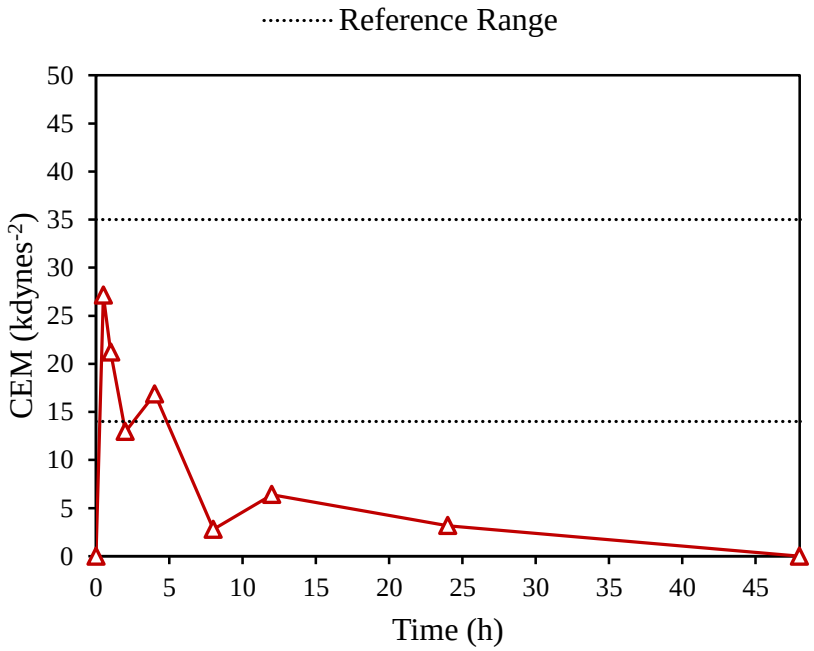
PCF PK/PD Linear Model Time Dependent Profile



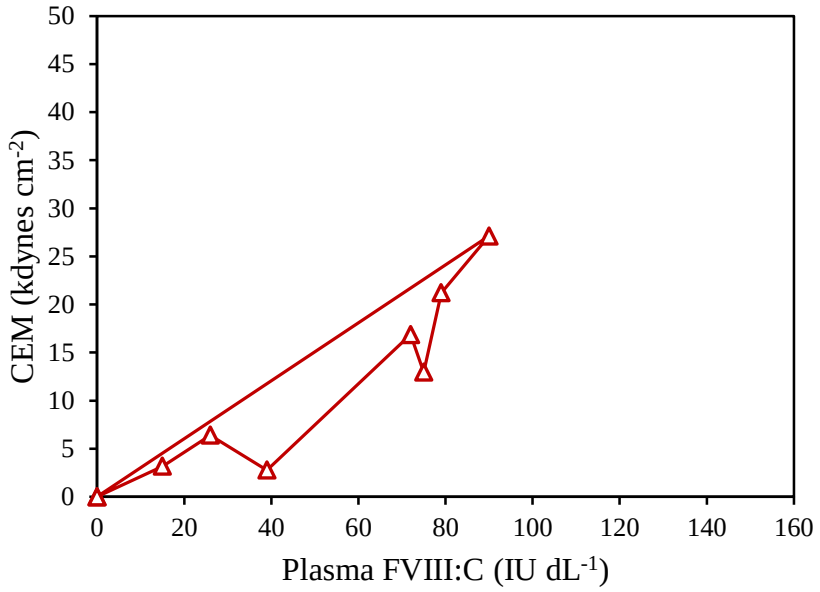
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



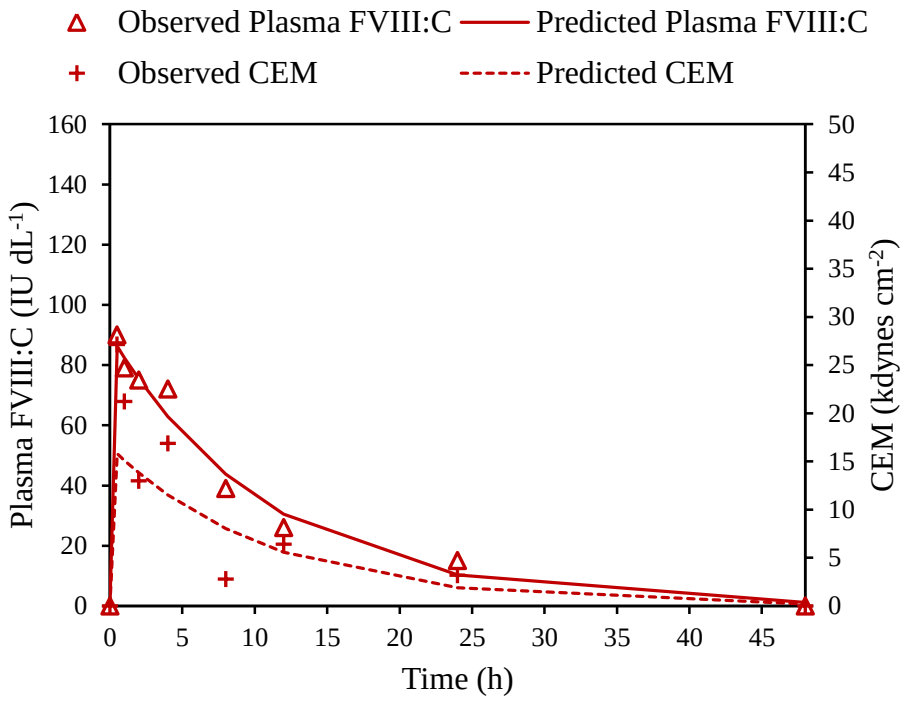
CEM-Time Profile



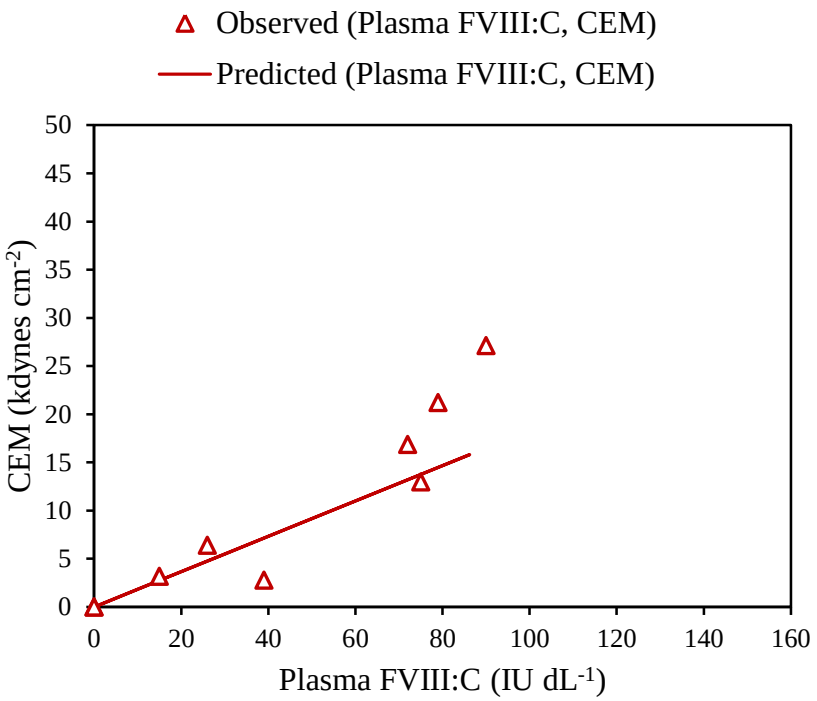
CEM-Plasma FVIII:C Profile



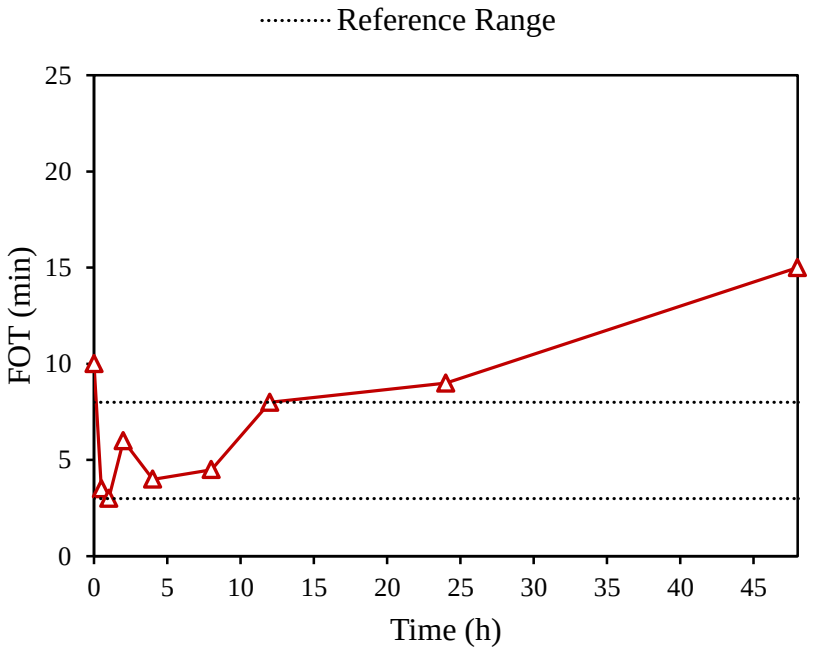
CEM PK/PD Linear Model Time Dependent Profile



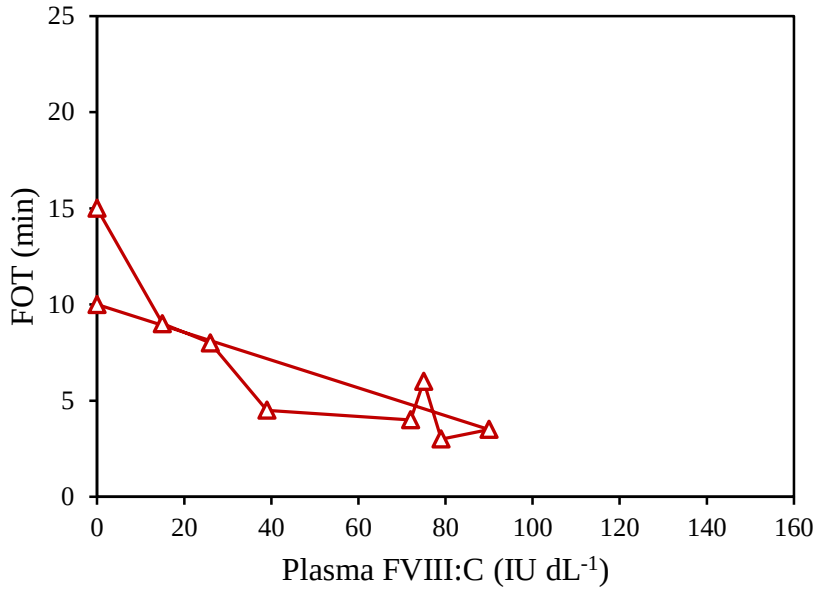
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



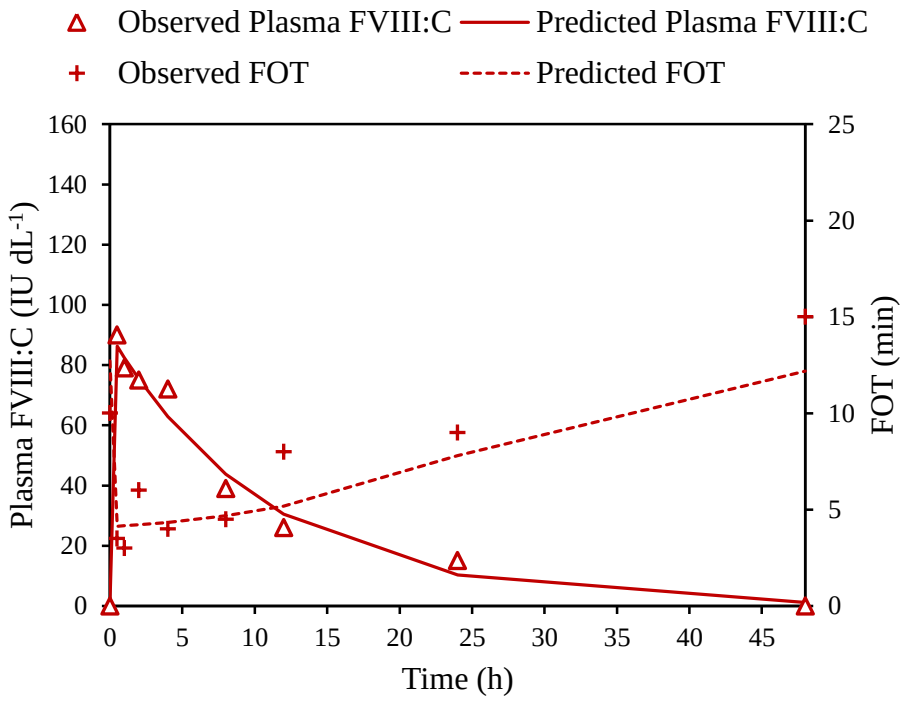
FOT-Time Profile



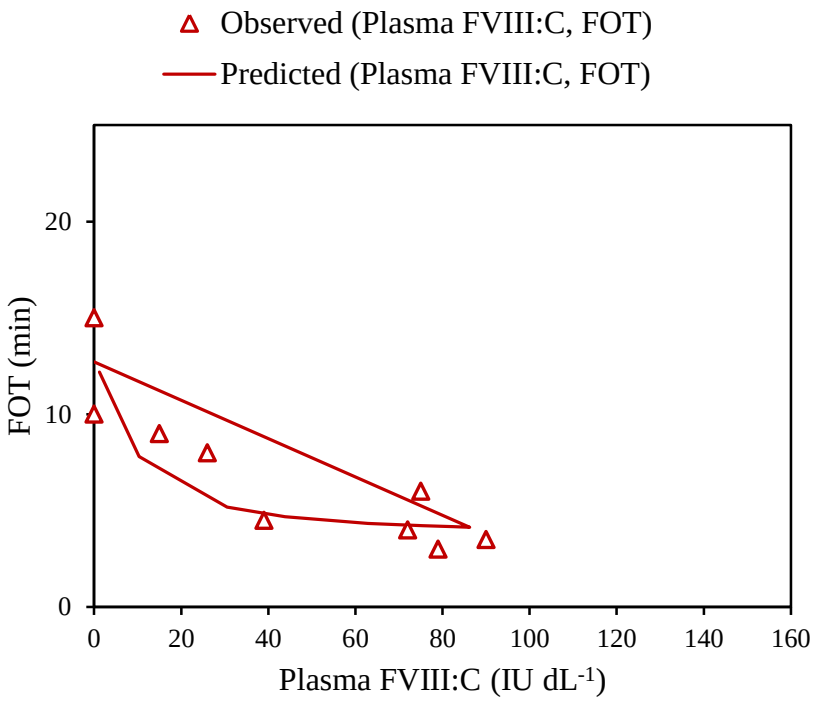
FOT-Plasma FVIII:C Profile



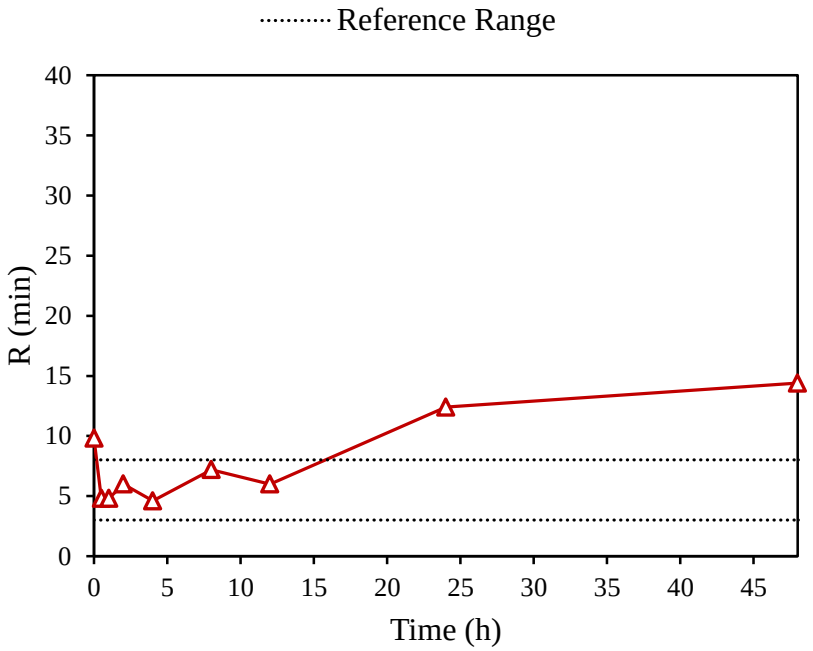
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



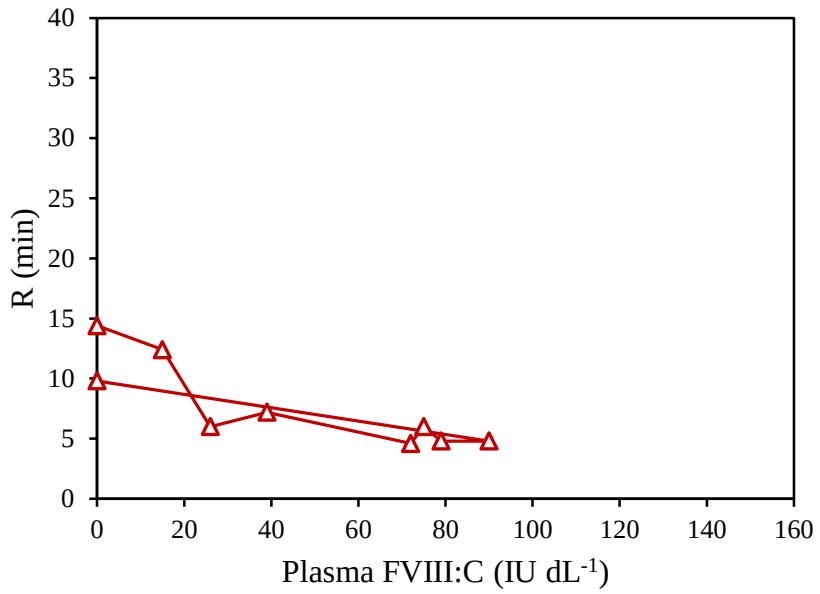
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



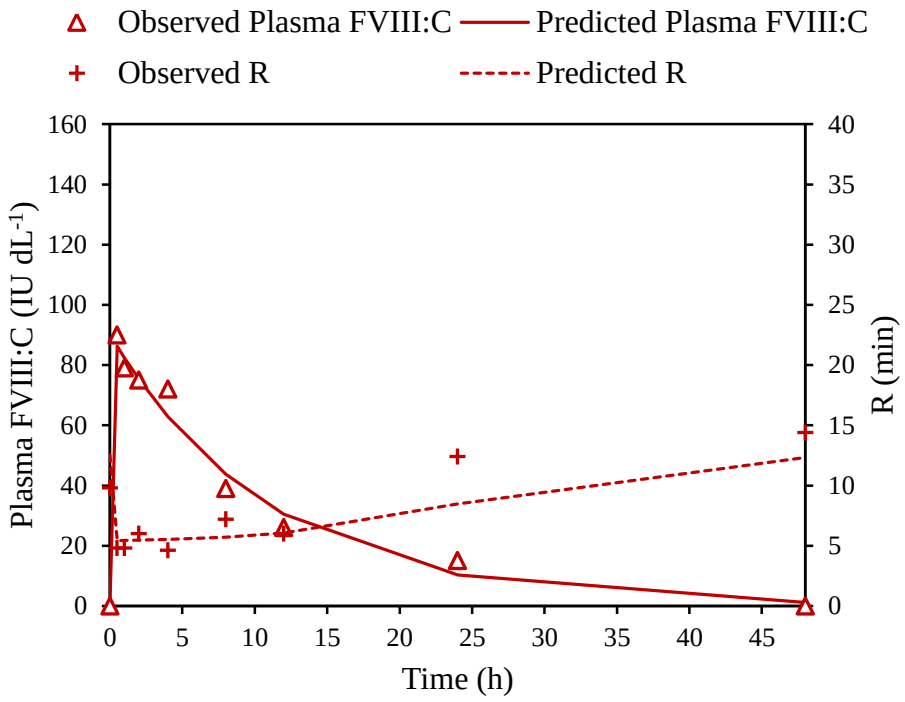
R-Time Profile



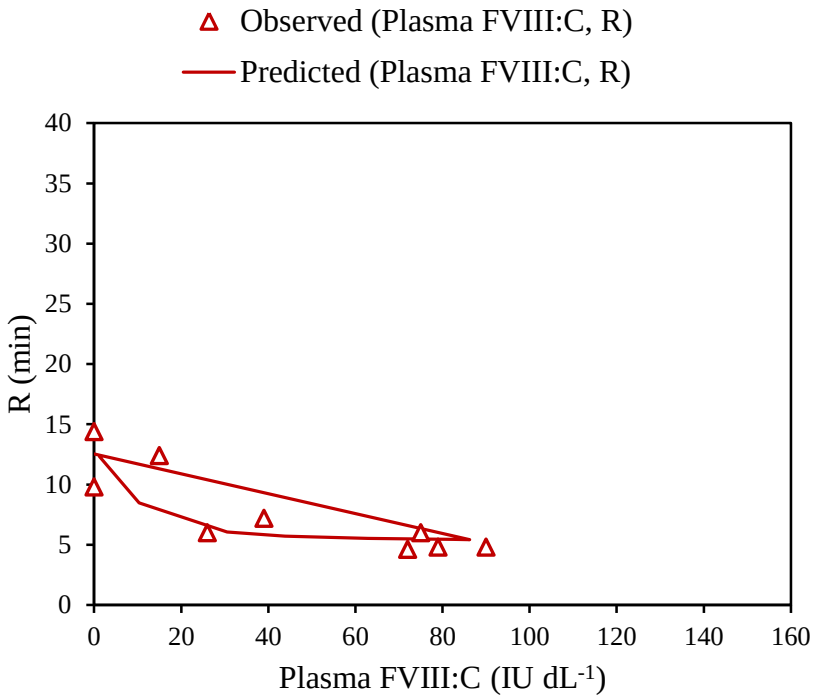
R-Plasma FVIII:C Profile



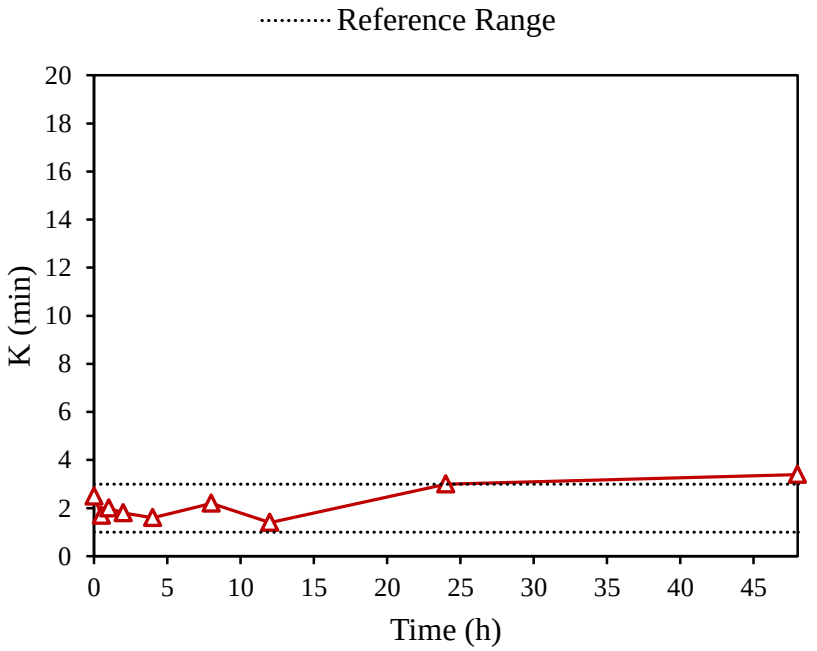
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



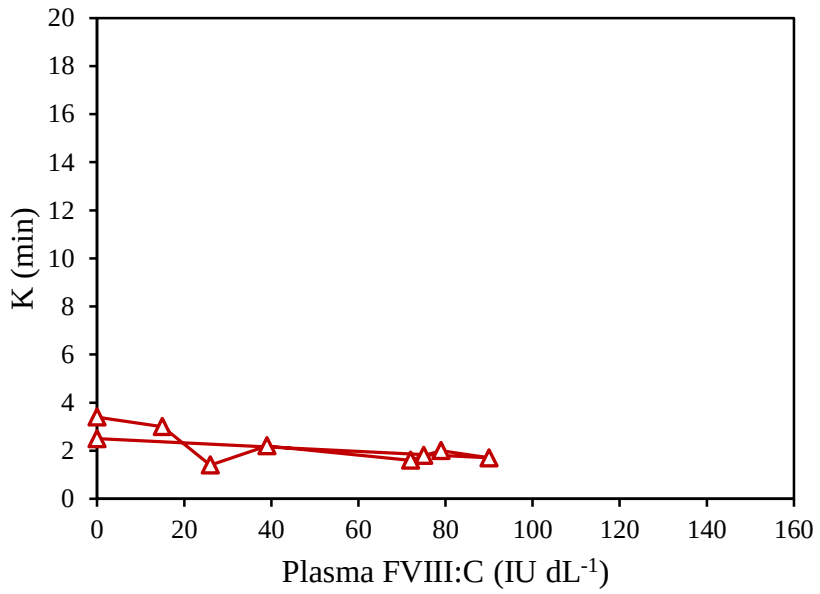
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



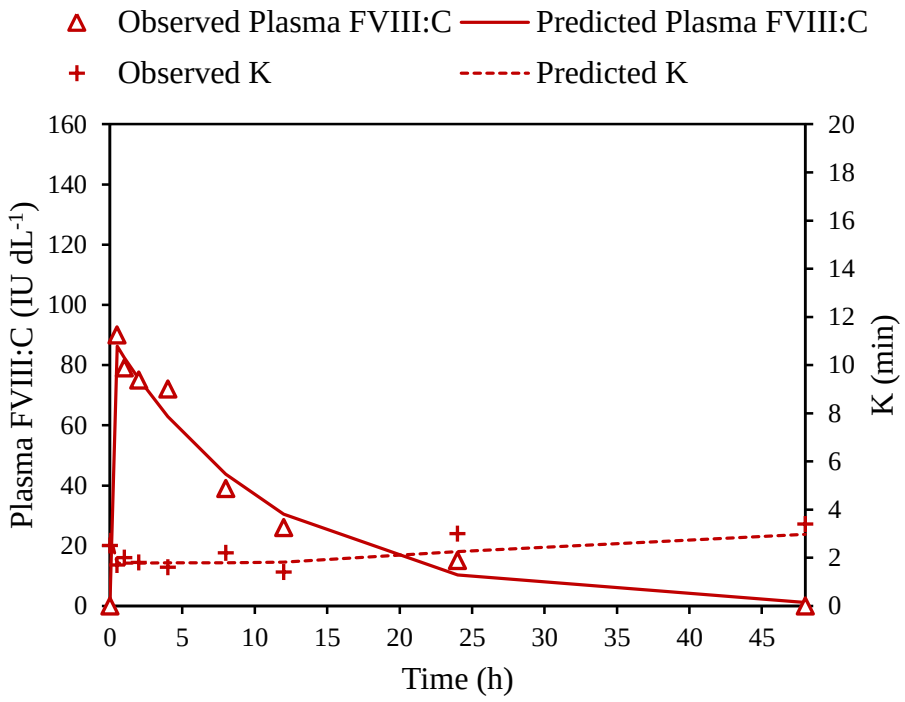
K-Time Profile



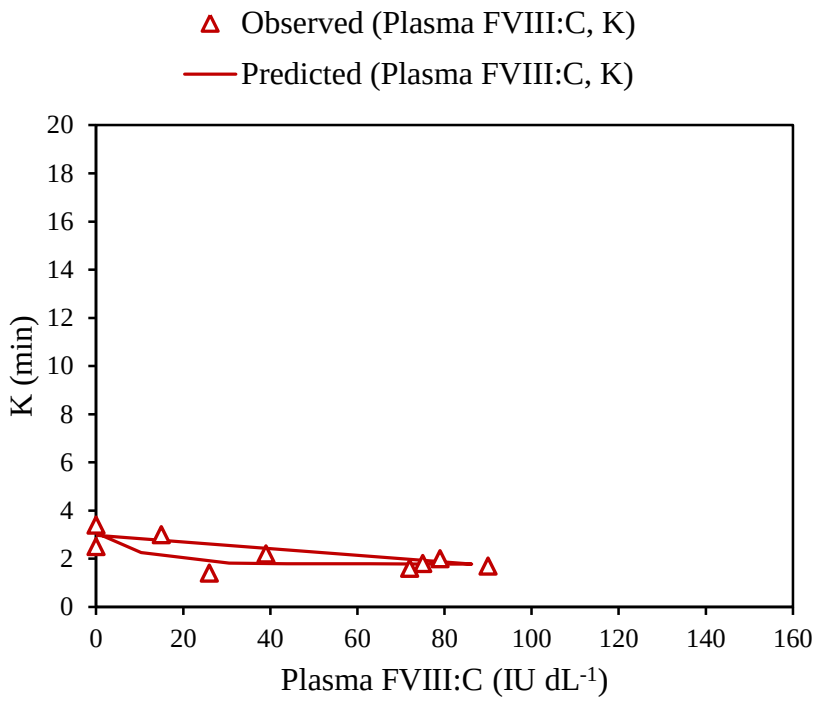
K-Plasma FVIII:C Profile



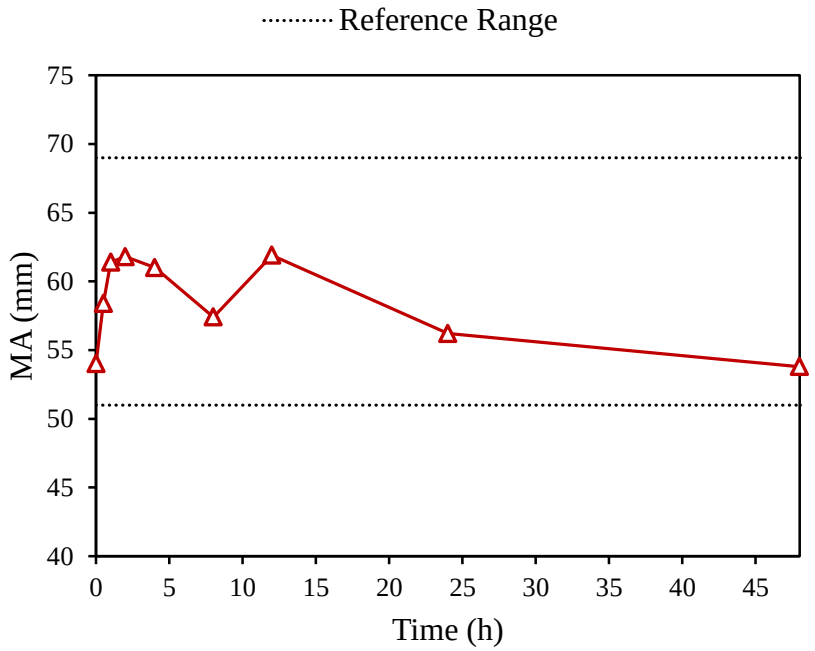
K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile

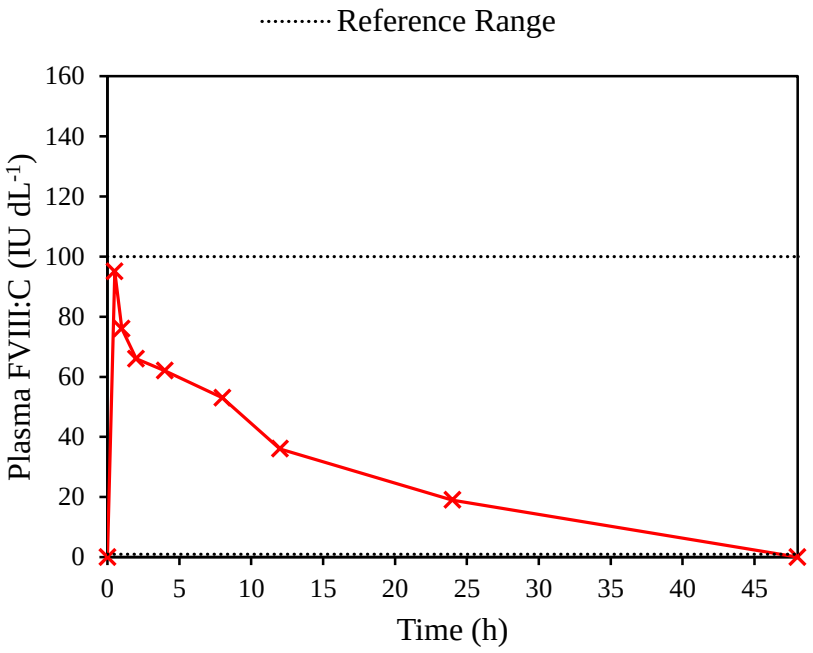


MA-Time Profile

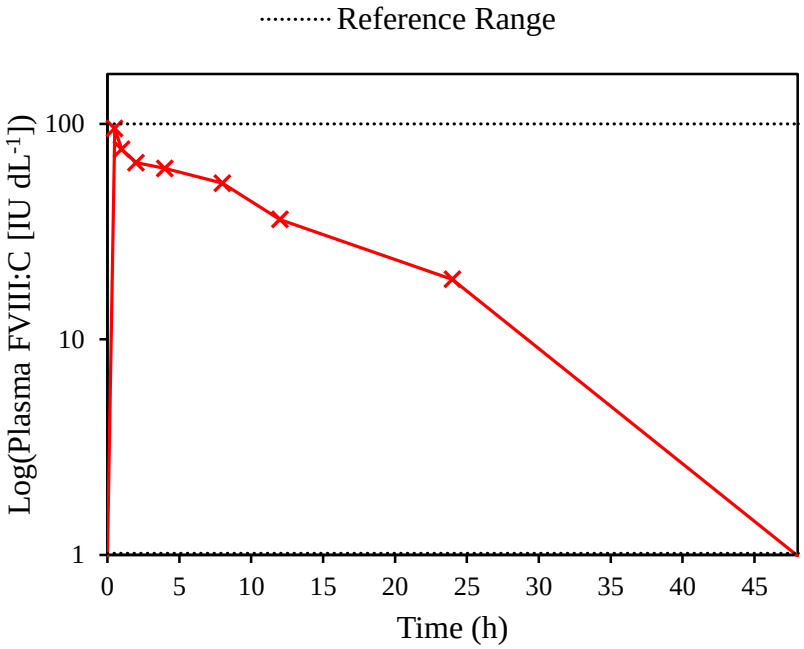


Appendix 13. Individual Profiles for Patient 9

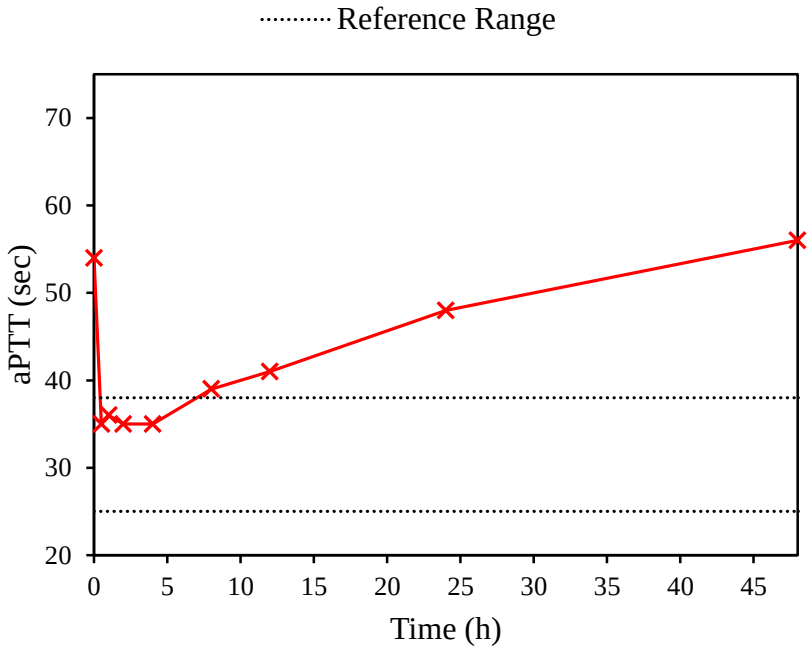
Plasma FVIII:C-Time Profiles



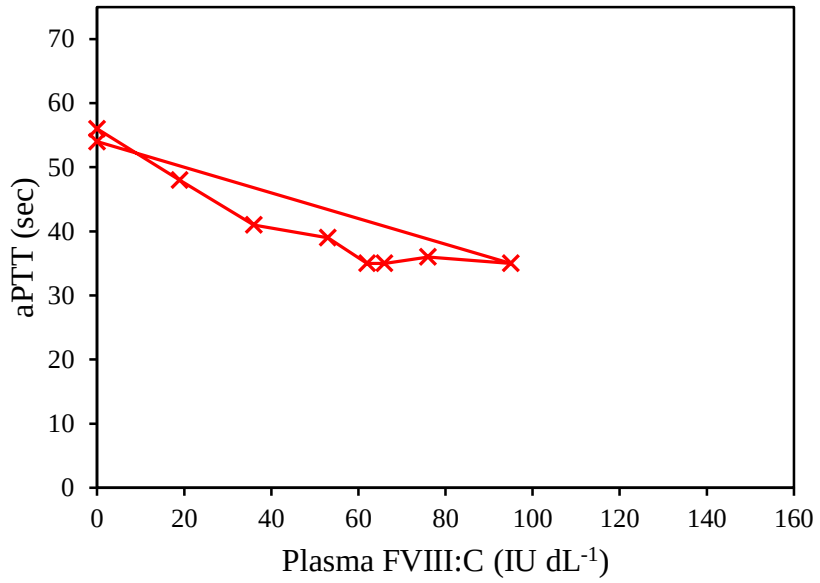
Plasma FVIII:C-Time Profiles on a Logarithmic Scale



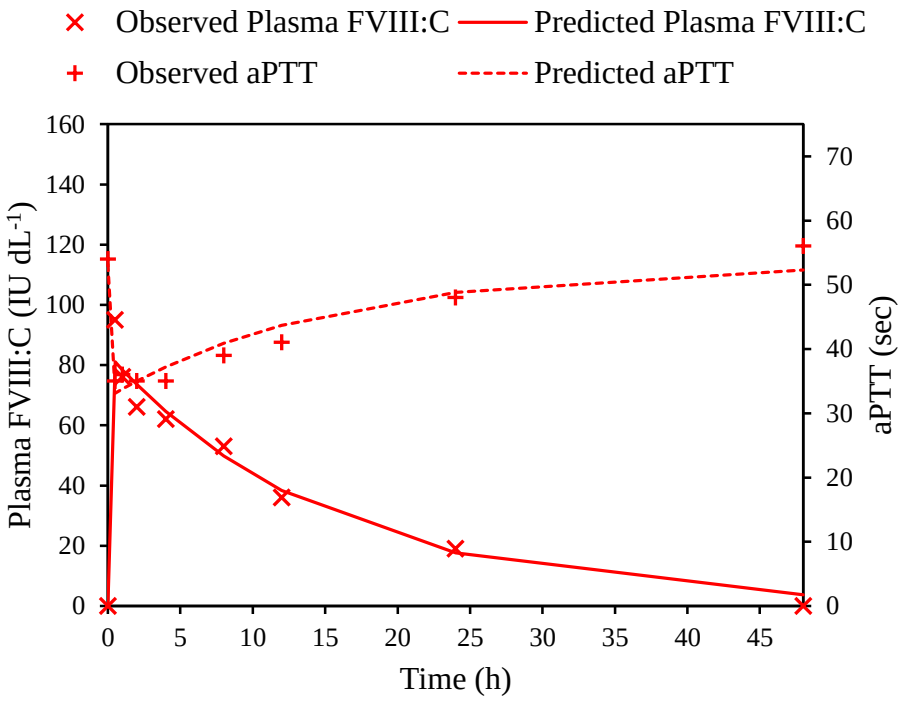
aPTT-Time Profile



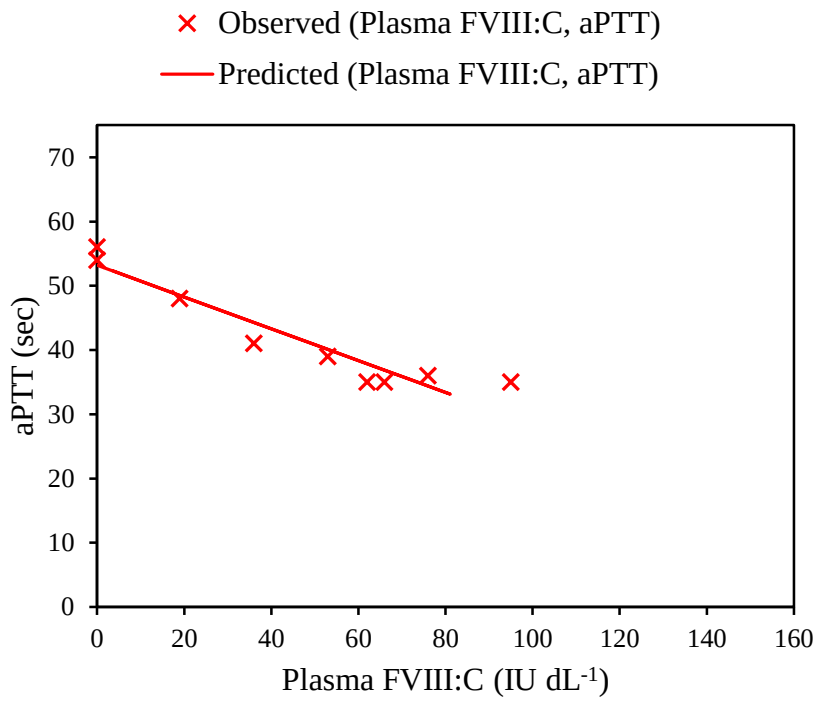
aPTT-Plasma FVIII:C Profile



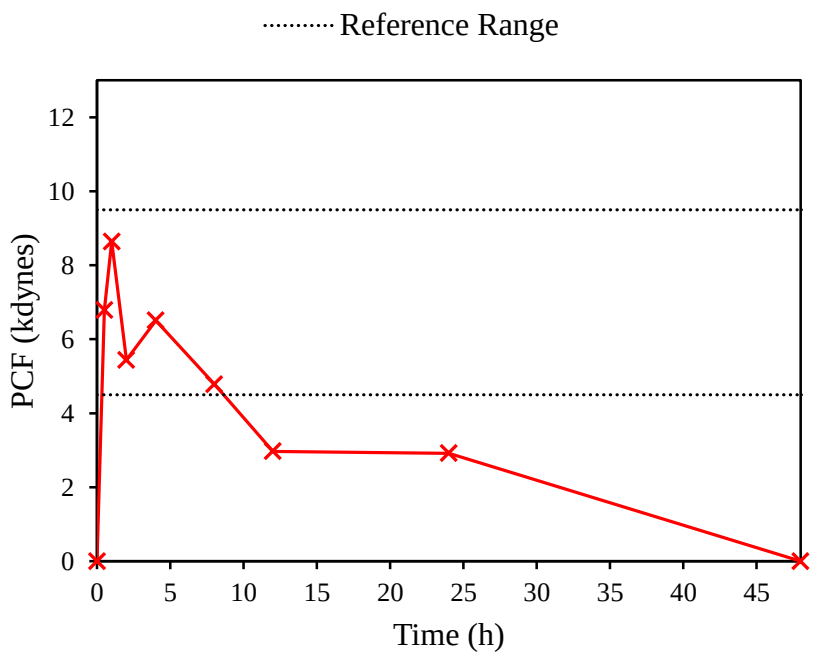
aPTT PK/PD Linear Model Time Dependent Profile



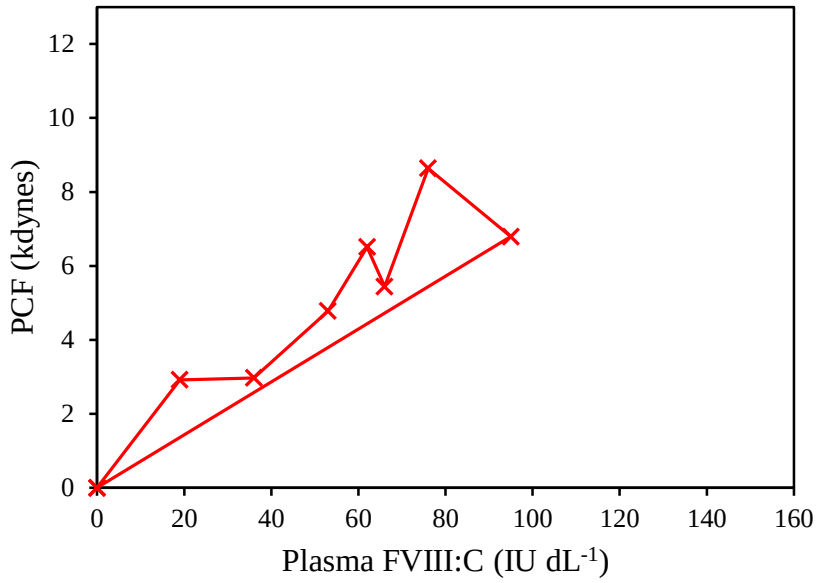
aPTT PK/PD Linear Model Plasma FVIII:C Dependent Profile



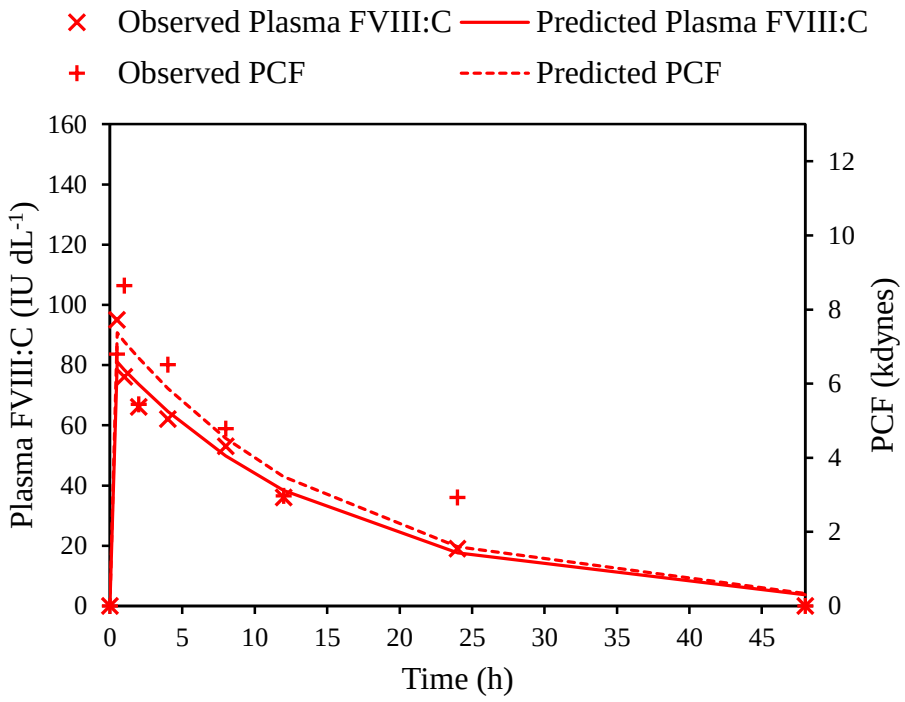
PCF-Time Profile



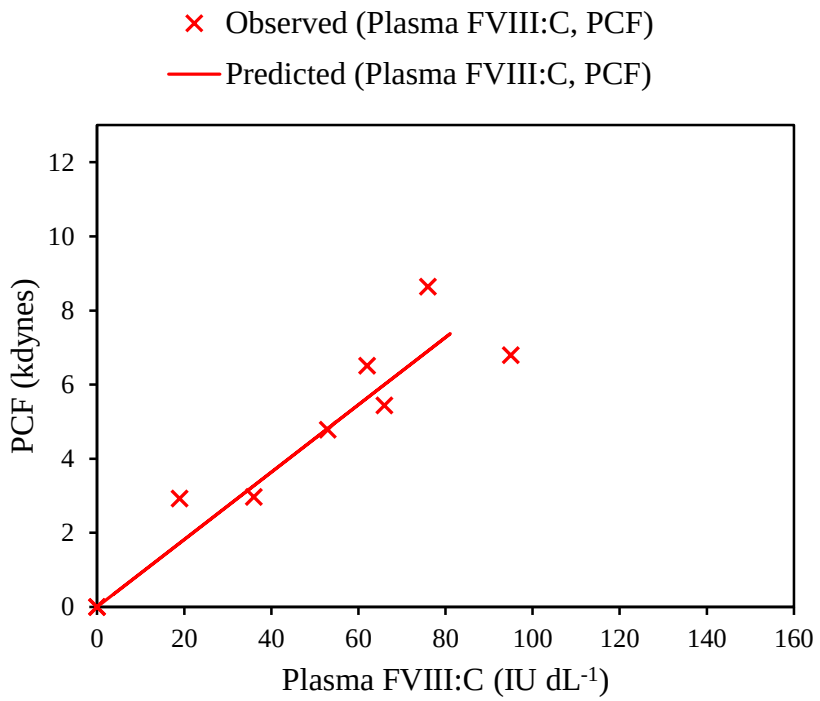
PCF-Plasma FVIII:C Profile



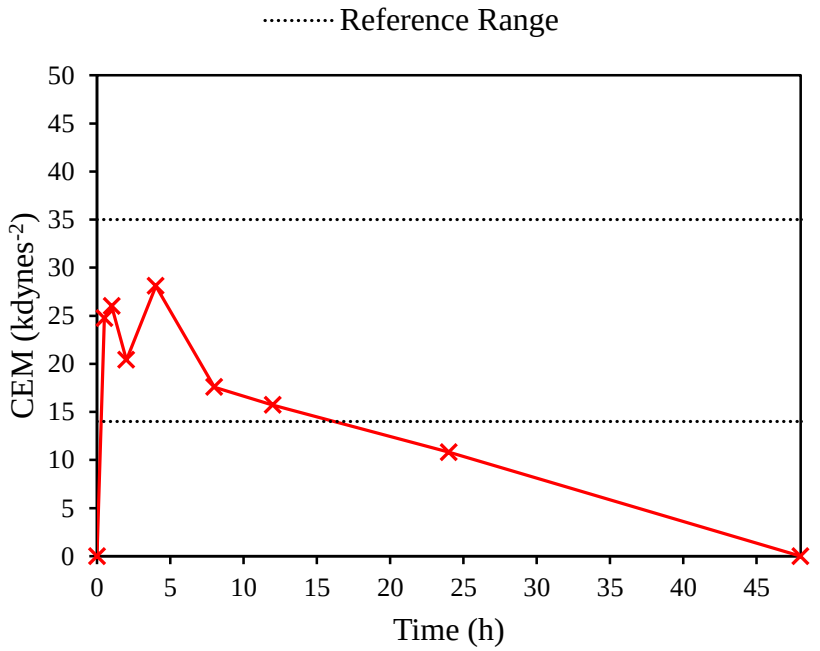
PCF PK/PD Linear Model Time Dependent Profile



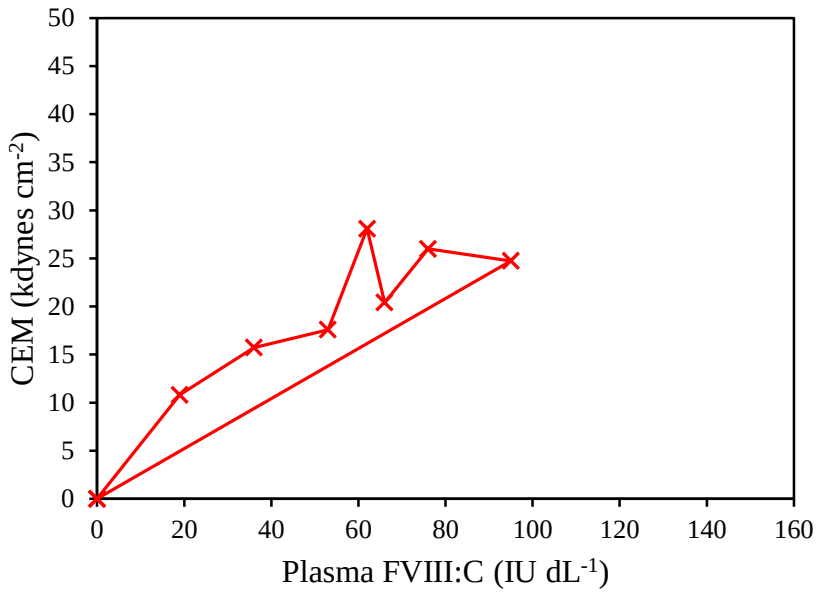
PCF PK/PD Linear Model Plasma FVIII:C Dependent Profile



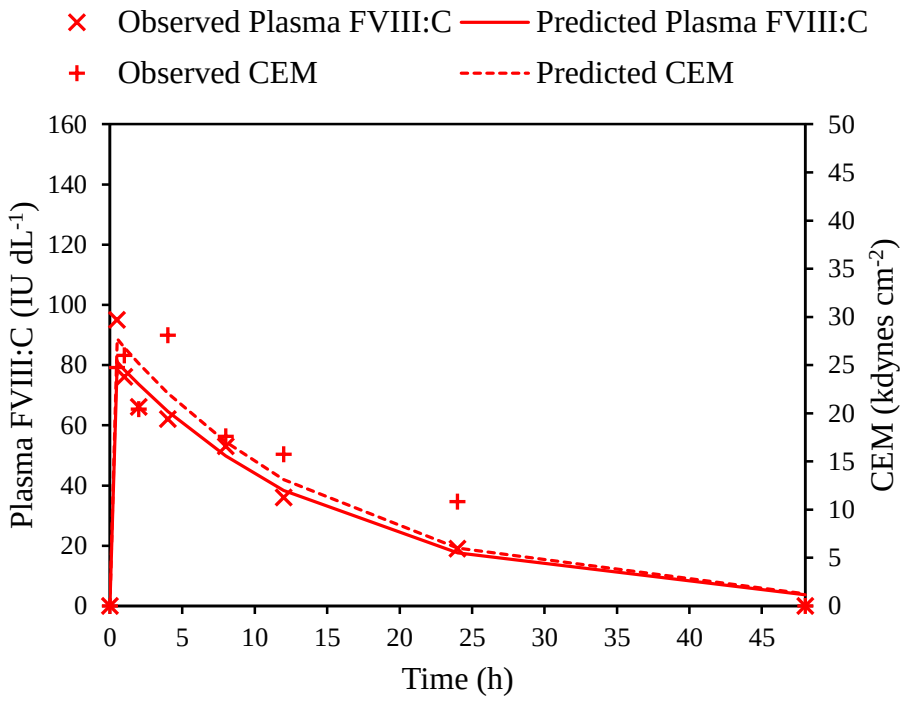
CEM-Time Profile



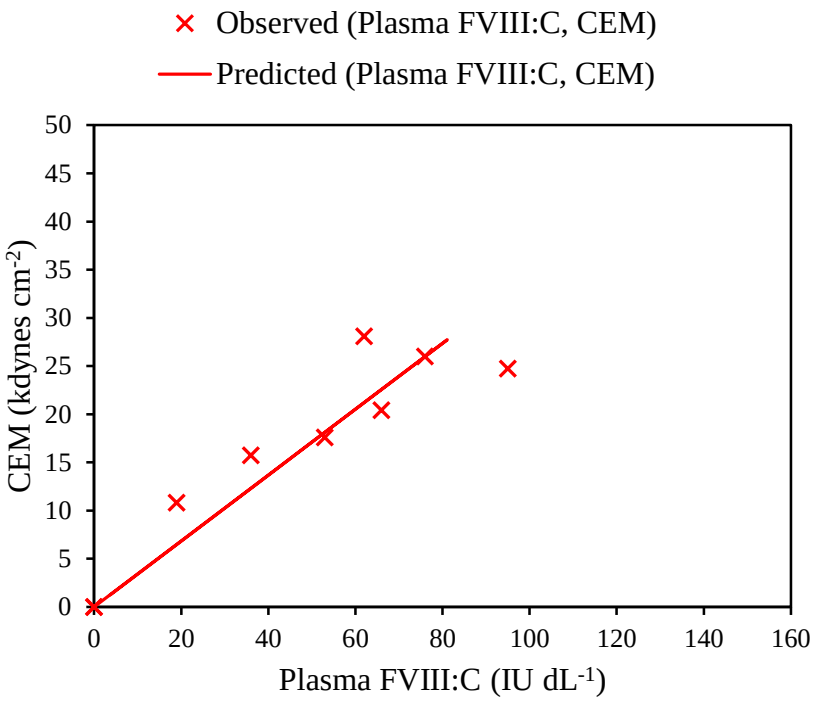
CEM-Plasma FVIII:C Profile



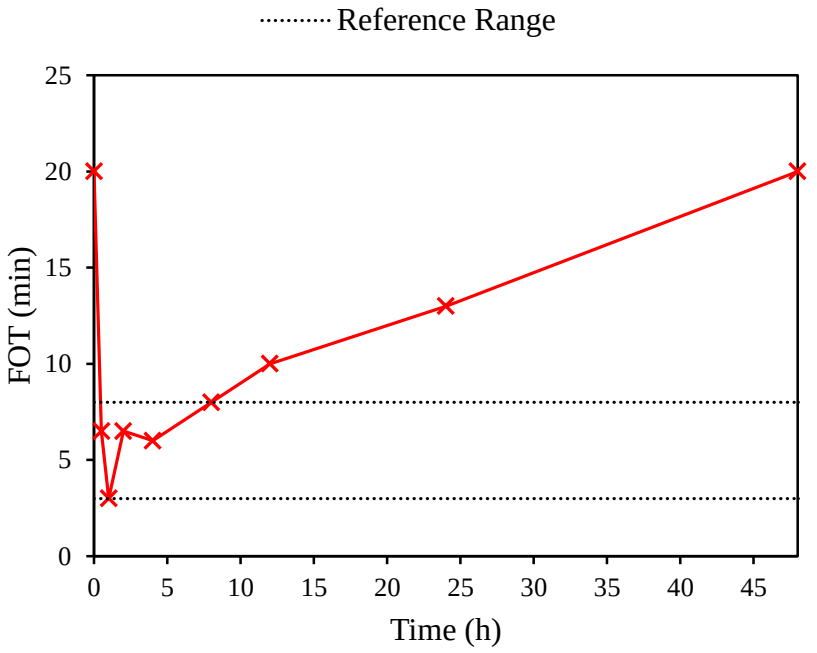
CEM PK/PD Linear Model Time Dependent Profile



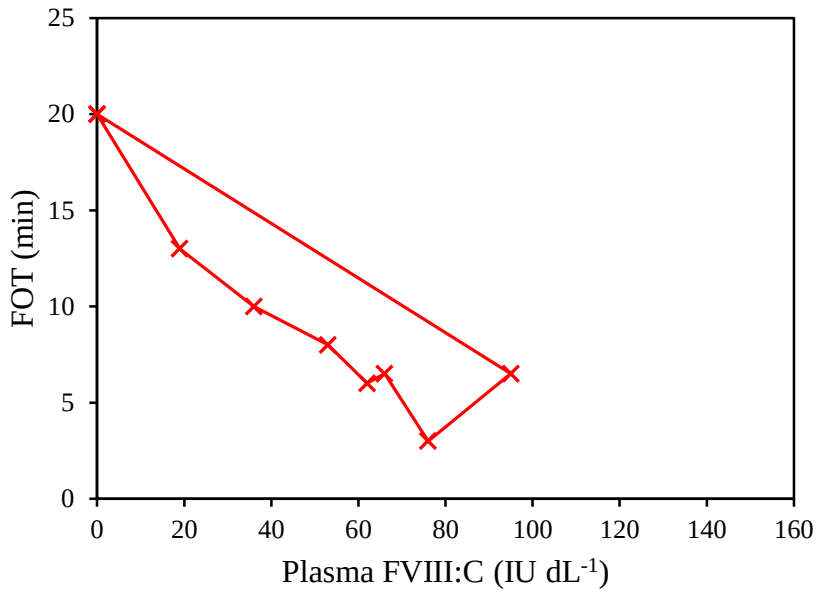
CEM PK/PD Linear Model Plasma FVIII:C Dependent Profile



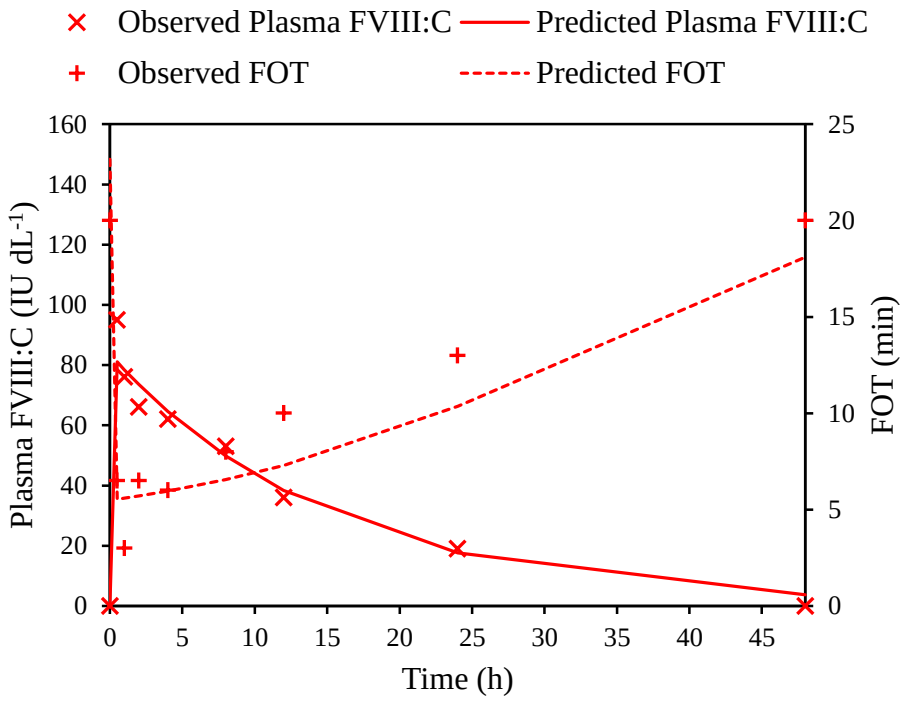
FOT-Time Profile



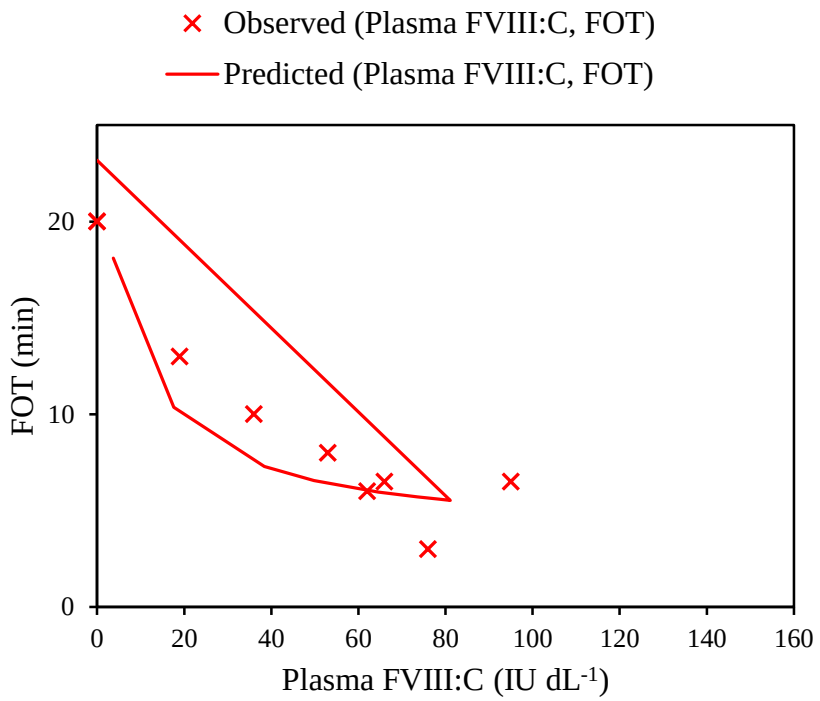
FOT-Plasma FVIII:C Profile



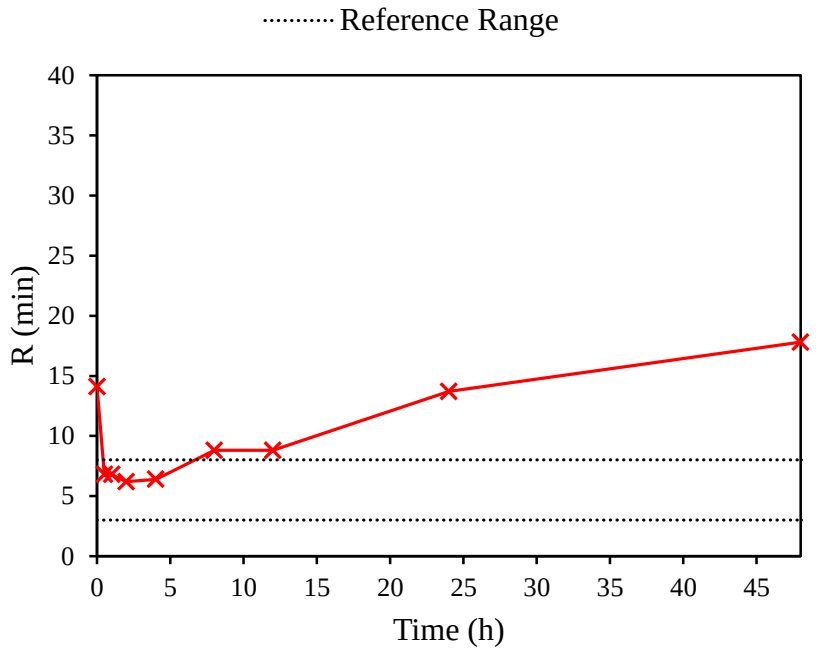
FOT PK/PD Sigmoidal E_{max} Model Time Dependent Profile



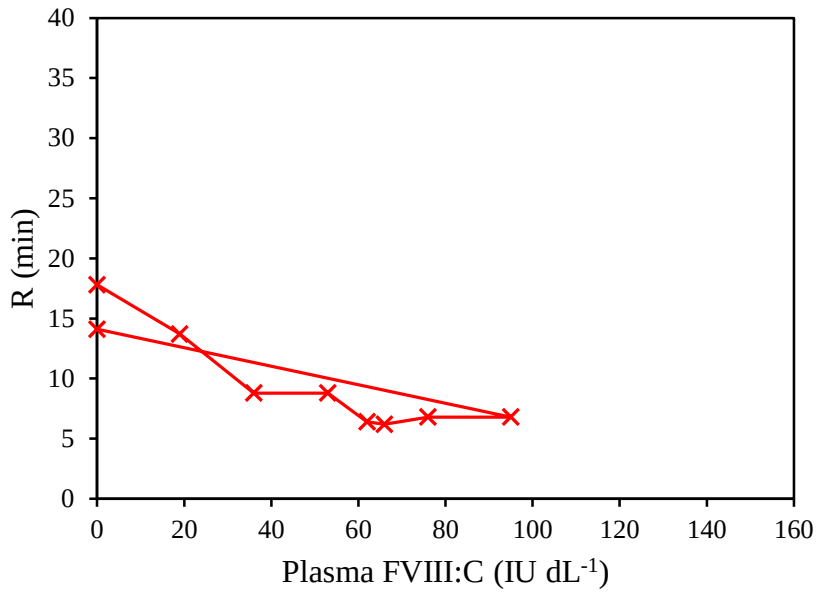
FOT PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



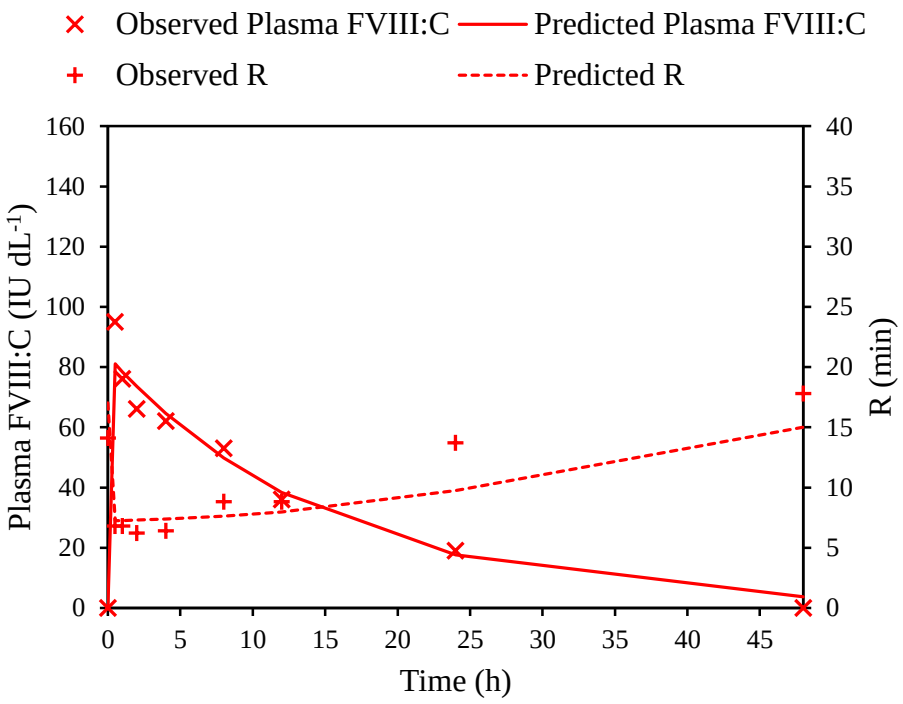
R-Time Profile



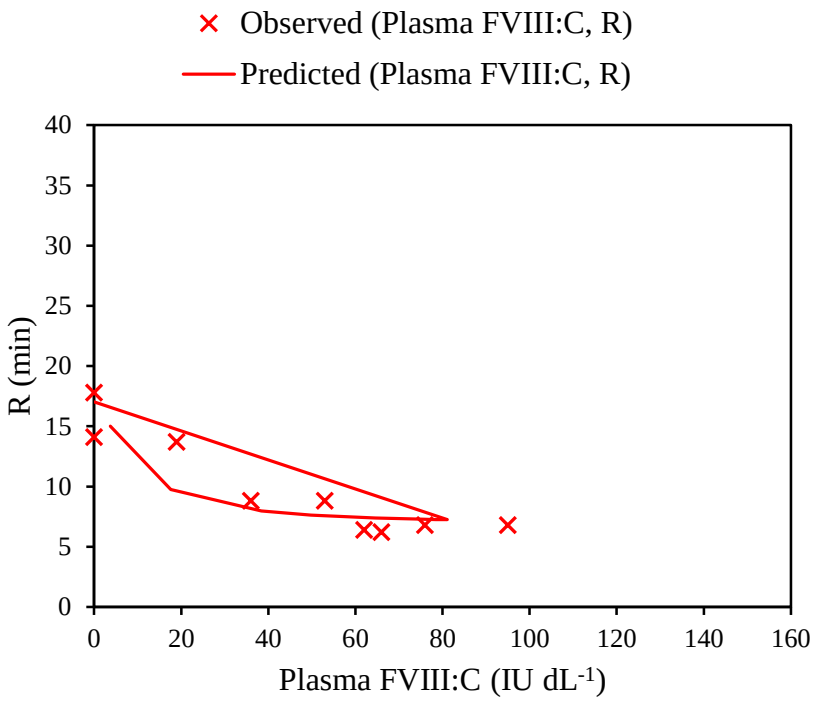
R-Plasma FVIII:C Profile



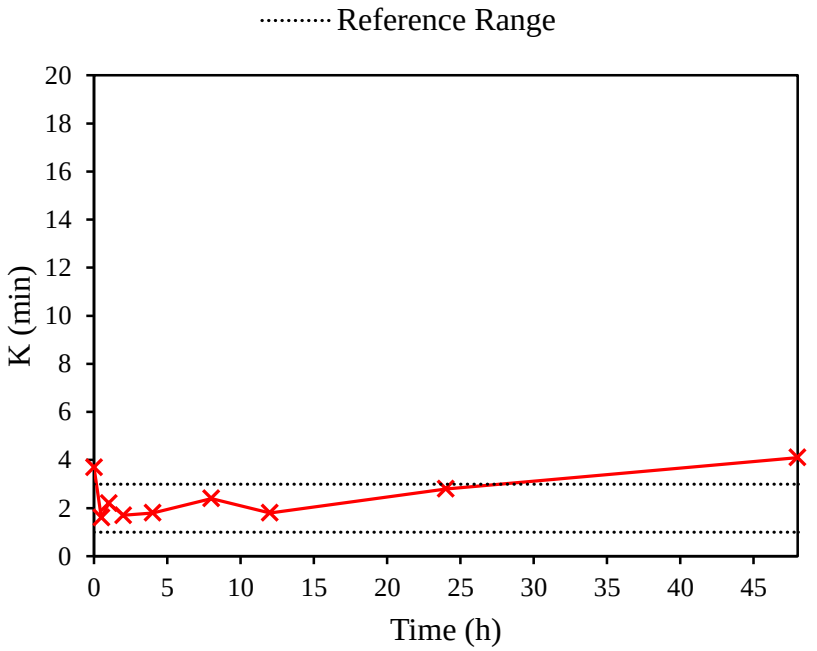
R PK/PD Sigmoidal E_{max} Model Time Dependent Profile



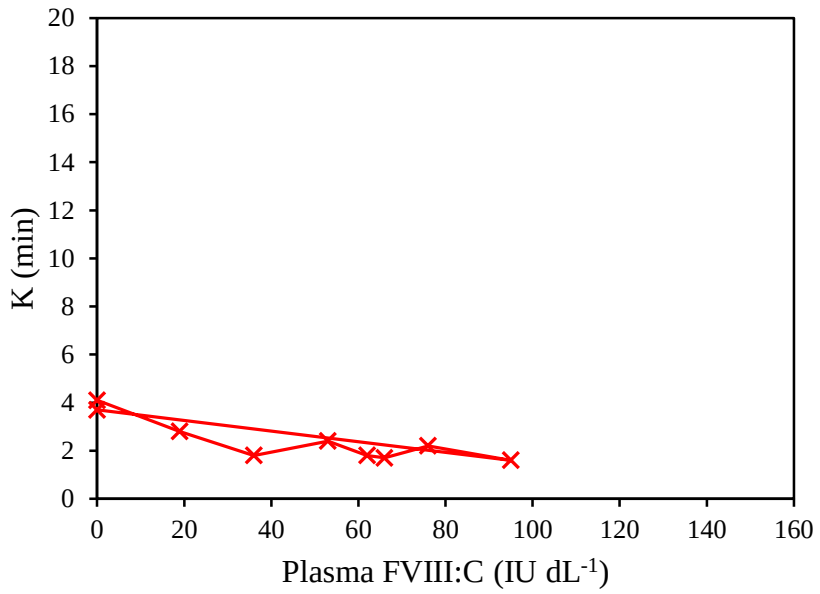
R PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



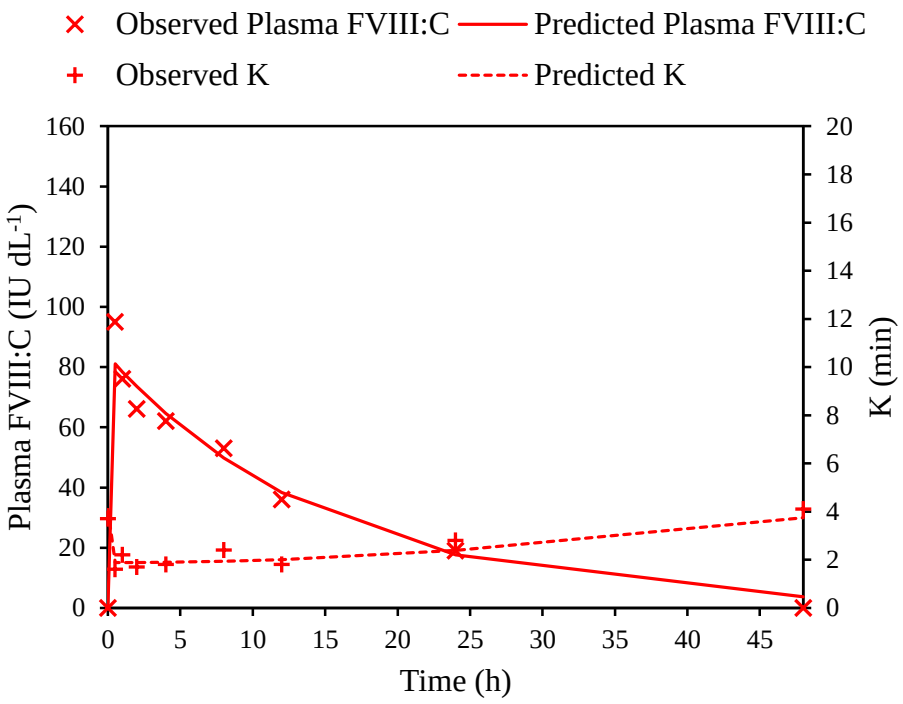
K-Time Profile



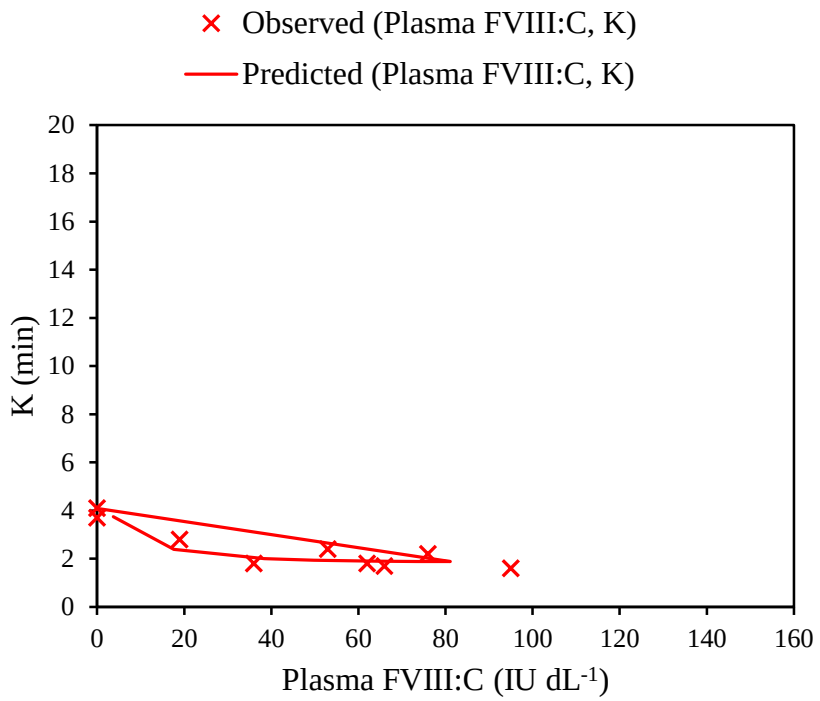
K-Plasma FVIII:C Profile



K PK/PD Sigmoidal E_{max} Model Time Dependent Profile



K PK/PD Sigmoidal E_{max} Model Plasma FVIII:C Dependent Profile



MA-Time Profile