

# CLINICAL PHARMACOLOGY REVIEW

Division of Hematology  
Office of Blood Review & Research

STN 125251/0

Product: Wilate

Sponsor: Octapharma Pharmazeutica Produktionsges. M.b.H.

Proposed Indication: For treatment ----(b)(4)---- of spontaneous -----(b)(4)----- bleedings in patients with von Willebrand disease (VWD)

Date Received: June 4, 2009

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Through: Mahmood Farshid, Ph.D.

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## INTRODUCTION

On December 14, 2006, Octapharma submitted to the United States Food and Drug Administration (US FDA) their product ‘Wilate’ for a marketing license in the USA. The submission consisted of several pharmacokinetic (PK) studies but the pivotal PK study was Wilate-12. The pharmacokinetic data (plasma concentrations vs time) of Wilate generated by the then analytical method (BCS Standard method) were not interpretable. The analytical method was not sensitive enough to appropriately detect Wilate concentrations (VWF:RCo) in plasma. According to the sponsor, the method was somewhat ‘coarse’ and the results were limited to one of a discrete series of possible concentrations, 1%, 6%, 12%, 24%, and 36%, etc. The end result of this analytical method was that following administration to VWD, measured plasma concentrations of VWF:RCo remained unchanged in many subjects during multiple sampling at different time points. Since these concentrations might not be accurate, the PK parameters

generated from these concentrations versus time data might also be inaccurate. Therefore, the FDA recommended that the sponsor develop a sensitive analytical method to measure the relevant concentrations of Wilate in plasma and then conduct a PK study with a suitable sample size or reanalyze the blood samples of Wilate with the newly developed analytical method.

The current report presents a re-analysis of the patient samples from the comparative crossover study (WIL-12) and seeks to overcome the validity issues expressed by the FDA with respect to the analytical assay. The study batch potencies and patient plasma concentrations were reassessed in retention samples from study WIL-12 by the use of an improved assay methodology.

The purpose of this analytical method is to carry out the quantitative assay of the activity of von Willebrand factor:ristocetin cofactor (VWF:RCo) in the plasma samples of patients in clinical study WIL-12. All plasma samples were assayed in the same laboratory by one laboratory technician. The same material lots were used for all the measurements.

The current analytical method is based on the agglutination of thrombocytes in the presence of VWF:RCo and the antibiotic ristocetin A. In the presence of ristocetin, the von Willebrand-Factor of the sample causes an agglutination of the stabilized platelets contained in the von Willebrand-reagent. The occurring agglutination reduces the turbidity of the sample. The change of the optical density at 570 nm is measured by the Behring Coagulation System (BCS) XP and based on the calibration curve, automatically converted into IU/mL.

A fully automated VWF:RCo assay performed on the Siemens BCS-XP machine, was used in the re-analysis. This included a modification of the standard Siemens method. Results from both standard and modified assay methods are reported here. A brief description of the assay methods are as follows:

The patient data from the WIL-12 study was assayed using the standard method as described for VWF:RCo on the BCS by the manufacturer (Siemens) and a modification of this method as developed in the -----(b)(4)-----. The automated standard BCS assay for VWF:RCo by Siemens is cost effective and has analytical superiority, at least when samples with normal or near normal activities are analyzed, compared to the manual aggregometer-based assay. However, the detection limit is far from optimal and therefore; of limited use when patients with VWD are phenotyped in the laboratory. Furthermore, the standard BCS assay is not suited for estimation of samples with VWF containing concentrates as it tends to underestimate the VWF:RCo activities in samples containing VWF concentrates compared to the assigned potencies that had been determined with a manual assay. The standard BCS assay may also overestimate the activity when samples with low VWF content are analyzed due to a poor sensitivity in the low measuring range which makes the assay unsuitable for PK measurements. The problems with the standard BCS assay are also clearly illustrated in this study where the potencies of both Wilate and Humate-P showed a large difference from the originally labeled VWF:RCo potencies. This resulted in low VWF:RCo to VWF:Ag for both Wilate and Humate-P lots of only 0.65 and 0.72 respectively. The PK profiles for the two concentrates also illustrated the above mentioned shortcomings of the standard BCS method with low recovery in the higher measuring range but

with higher dilution the recoveries are increased and finally higher than the corresponding recoveries for the other VWF parameters.

The modified VWF:RCo assay was developed to overcome the shortcomings of the standard BCS assay. In order to obtain a better correlation between manual and automated assays the -----(b)(4)----- . To improve and extend the measuring range, particularly in the low end, -----(b)(4)-----  
-----  
----- . This makes it possible for a level of detection as low as of ---(b)(4)--. Thus, with a combination of -----(b)(4)-----  
----- an automated assay with improved characteristics was obtained. This modified assay has the potential to be used for both VWD diagnosis and for monitoring of patients on substitution therapy. The modified VWF:RCo assay has been extensively validated and the results from the WIL-12 study further supports the suitability of this assay protocol for PK-profiling purposes. The VWF:RCo potencies obtained with the modified assay are also in better agreement with the originally labeled values, which in turn revealed a VWF:RCo to VWF:Ag ratio closer to 1 compared with the standard BCS assay. When used to calculate the actual doses to study subjects the modified assay appeared higher, as compared to the standard BCS assay.

The pharmacokinetic review of Wilate is based on the modified as well as standard BCS analytical method.

## CLINICAL PHARMACOLOGY LABELING

### 12. CLINICAL PHARMACOLOGY

#### 1.1 Mechanism of Action

VWF and FVIII are normal constituents of human plasma. VWF is a multimeric protein with two key functions. It is an adhesive molecule, which mediates the binding between platelets and damaged sub-endothelial tissues. It is also a carrier protein, involved in the transport and stabilization of FVIII. Patients suffering from VWD have a deficiency or abnormality of VWF. This reduction in VWF concentration in the bloodstream results in a correspondingly low FVIII activity and an abnormal platelet function, resulting in excessive bleeding. The VWF in Wilate<sup>®</sup> is derived from a normal constituent of the human plasma and is expected to behave in the same way as endogenous VWF. Thus, administration of VWF allows correction of the hemostatic abnormalities in VWD patients at two levels:

- The VWF re-establishes platelet adhesion to the vascular sub-endothelium at the site of vascular damage (as it binds both to the vascular sub-endothelium and to the platelet membrane), providing primary hemostasis, as shown by the shortening of the bleeding time. This effect occurs immediately
- The VWF induces correction of the associated FVIII deficiency in VWD. Administered intravenously, VWF binds endogenous FVIII (which is produced normally by the patient), and by stabilizing this factor, avoids its rapid degradation. This action is slightly delayed. However, administration of a FVIII-containing VWF preparation like Wilate<sup>®</sup> rapidly restores the FVIII activity level to normal immediately.

#### 1.2 Pharmacodynamics

There have been no specific pharmacodynamic studies on Wilate.

#### 1.3 Pharmacokinetics

An open label, prospective, randomized, controlled, two-arm cross-over Phase 2 study with Wilate<sup>®</sup> and a comparator product was conducted at 6 sites in the US. In this study, pharmacokinetic (PK) profiles of Wilate<sup>®</sup> were determined for FVIII:C, VWF:RCo, VWF:Ag, and VWF:CB. Each of twenty-two subjects with inherited VWD [Type 1, n=6; Type 2, n=9 (6 Type 2A, 1 Type 2B, and 2 Type 2M); and Type 3, n=7] received an intravenous bolus dose of Wilate<sup>®</sup> containing approximately 40 IU of VWF:RCo/kg BW. PK parameters of VWF:RCo and FVIII are summarized in Table 1 and Table 2, respectively.

**Table 1 Pharmacokinetic Parameters of VWF:RCo: mean  $\pm$  sd (range)**

Parameters	VWD type I (n = 5)	VWD type II (n = 9)	VWD type III (n = 6)	Total (n = 20)
C <sub>max</sub> (IU/dL)	74 $\pm$ 13 (62-91)	77 $\pm$ 18 (40-100)	79 $\pm$ 13 (65-102)	76 $\pm$ 15 (40-102)
AUC <sub>(0-inf)</sub> (IU*hr/dL)	1633 $\pm$ 979 (984-3363)	1172 $\pm$ 421 (571-1897)	995 $\pm$ 292 (527-1306)	1235 $\pm$ 637 (527-3363)
Half-life (hrs)	24.7 $\pm$ 17.9 (11.2 - 48.5)	15.3 $\pm$ 6.3 (6.0 - 26.4)	9.1 $\pm$ 2.6 (5.7 - 12.9)	15.8 $\pm$ 11.0 (5.7 - 48.5)
CL (mL/h/kg)	3.1 $\pm$ 1.1 (1.2 - 4.1)	4.1 $\pm$ 1.7 (2.0 - 7.1)	4.2 $\pm$ 1.4 (3.0 - 6.6)	3.7 $\pm$ 1.5 (1.8 - 8.8)
V <sub>ss</sub> (mL/kg)	81.7 $\pm$ 38.5 (15.3-74.2)	76.6 $\pm$ 35.4 (45.3-158.8)	49.4 $\pm$ 16.7 (29.7-67.1)	69.7 $\pm$ 33.2 (29.7-158.8)
MRT (hrs)	32.7 $\pm$ 25.8 (15.3 - 74.2)	19.7 $\pm$ 5.6 (9.9 - 27.1)	11.9 $\pm$ 2.9 (9.2 - 15.9)	17.4 $\pm$ 4.5 (10.2 - 28.8)
Recovery (%IU/kg)	1.8 $\pm$ 0.2 (1.5 - 2.0)	1.8 $\pm$ 0.5 (1.0 - 2.4)	2.1 $\pm$ 0.3 (1.8 - 2.6)	2.0 $\pm$ 0.5 (1.0 - 2.7)

CL = clearance; V<sub>ss</sub> = volume of distribution at steady state; MRT = mean residence time

The PK parameters reported in Table 4 are based on VWF:RCo values obtained using a modified Behring Coagulation System (BCS) analytical method. The measured concentrations (IU VWF:RCo/mL) are higher by the modified BCS than by the standard BCS analytical method which is used in some clinical laboratories. Dose adjusted C<sub>max</sub> and AUC determined by this modified BCS method are approximately 1.5 times higher than those by the standard BCS method. No difference has been found in incremental recovery.

**Table 2 Pharmacokinetic Parameters of FVIII:C: mean  $\pm$  sd (range) - chromogenic**

Parameters	VWD type I (n = 5)	VWD type II (n = 8*)	VWD type III (n = 6)	Total (n = 19*)
C <sub>max</sub> (IU/dL)	117.1 $\pm$ 12.1 (103-135)	147.2 $\pm$ 32.6 (102-206)	120 $\pm$ 23 (91-148)	112 $\pm$ 23 (59-148)
AUC <sub>(0-inf)</sub> (IU*hr/dL)	1187 $\pm$ 382 (523-1483)	1178 $\pm$ 1430 (544-4821)	2670 $\pm$ 854 (1874-3655)	2290 $\pm$ 1045 (464-4424)
Half-life (hrs)	17.5 $\pm$ 4.9 (10.9 - 23.8)	23.6 $\pm$ 8.3 (12.6 - 34.7)	16.1 $\pm$ 3.1 (11.8 - 20.1)	19.6 $\pm$ 6.9 (10.9 - 34.7)
CL (mL/h/kg)	4.4 $\pm$ 3.7 (2.5 - 11.0)	2.5 $\pm$ 0.9 (1.2 - 3.5)	2.0 $\pm$ 0.6 (1.4 - 2.8)	2.9 $\pm$ 2.1 (1.2 - 11.0)
V <sub>ss</sub> (mL/kg)	95.0 $\pm$ 53.8 (57.1 - 190.0)	79.5 $\pm$ 23.1 (52.8 - 116.2)	44.2 $\pm$ 10.4 (31.8 - 57.1)	72.4 $\pm$ 36.2 (31.8 - 190.0)
MRT (hrs)	24.1 $\pm$ 5.5 (17.2 - 31.5)	35.1 $\pm$ 14.2 (17.5 - 61.6)	23.0 $\pm$ 3.7 (18.0 - 27.7)	28.4 $\pm$ 11.1 (17.2 - 61.6)
Recovery (%IU/kg)	1.9 $\pm$ 0.5 (1.1 - 2.5)	2.2 $\pm$ 0.4 (1.6 - 2.8)	2.5 $\pm$ 0.5 (2.0 - 3.0)	2.2 $\pm$ 0.5 (1.1 - 3.0)

CL = clearance; V<sub>ss</sub> = volume of distribution at steady state; MRT = mean residence time

\*one subject with implausible long half-life not included in summary table, except for recovery result

**Effect of age and VWD type on the pharmacokinetics of Wilate<sup>®</sup>:**

Due to small sample size (in age or VWD type subsets) and high PK variability, it is difficult to conclude if age or type of VWD had any impact on the pharmacokinetics of Wilate.

**Effect of gender on the pharmacokinetics of Wilate<sup>®</sup>:**

Based on PK data of Wilate<sup>®</sup> from 8 males and 12 females, it appears that the females ( $4.35 \pm 1.54$  mL/hr/kg) had higher clearance of VWF:RCo than the males ( $3.16 \pm 1.19$  mL/hr/kg). The clinical significance of this finding is not known.

## COMMENTS

1. The modified analytical method for the measurement of VWF:RCo appears to be more sensitive than the standard BCS analytical method. The plasma concentrations-time data appear to follow a pattern (decline with time) with the modified analytical method than the BCS analytical method. This may be because at lower concentrations of VWF:RCo, the standard BCS is not as sensitive as the modified method. It also appears that the concentrations measured by BCS are at least by 1.75 times lower (calculated based on mean AUC and per mg dose) than the concentrations measured by the modified BCS method. The  $C_{\max}$  is 1.5 times lower (calculated based on mean  $C_{\max}$  and per mg dose) by BCS analytical method than by the modified BCS method.
2. Between the two assay methods (modified and standard BCS), the pharmacokinetic parameters are different. The mean  $C_{\max}$  and  $AUC_{(0-\infty)}$  (without dose adjustment) of Wilate ( $n = 20$ ) are 2 and 2.5-fold higher by the modified assay than standard BCS assay. When accounted for all 20 subjects, the mean half-life appears to be comparable by the two analytical methods but the half-life of Wilate in patients with VWD type 3 is almost 50% shorter by modified assay than the standard BCS assay. From Figure II, it is apparent that it is difficult to calculate half-life with accuracy for Wilate from the flat terminal part of concentrations-time curve using standard BCS method. In short, there is uncertainty about the PK parameters estimated from standard BCS assay method.
3. Considering that most of the laboratories use the standard method, the FDA should consider either changing the analytical method for the measurement of VWF:RCo (from standard BCS to modified method) or apply a correction factor. A correction factor however, may not be uniform across all concentrations of VWF:RCo. For example, two or three different correction factors may be needed for low, medium, and high concentrations of VWF:RCo. In short, a pragmatic solution should be sought to reconcile the differences in plasma concentrations of Wilate introduced by the two analytical methods. In the clinical setting the manual method will be used to monitor treatment with Wilate and should be sufficiently sensitive for this purpose.

## **RECOMMENDATION**

The pharmacokinetic study design and analysis of Wilate by modified analytical method is acceptable.

The sponsor has revised the clinical pharmacology labeling section of Wilate as suggested by the FDA and is acceptable.

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**Study Title:** A prospective, randomized, controlled, open-labeled, two-arm crossover study investigating the pharmacokinetic properties of Wilate and Humate-P in subjects with inherited von Willebrand disease.

**Objectives:** The primary objective of the study was to determine the half-life of Wilate in terms of the FVIII coagulant activity (FVIII:C), the ristocetin cofactor activity (VWF:RCo), VWF antigen (VWF:Ag), and collagen binding activity (VWF:CB) of Wilate and to compare these parameters with those for Humate-P. The secondary objectives of this study were to calculate the incremental recovery of FVIII:C, VWF:RCo, VWF:Ag, and VWF:CB; and to assess the safety and tolerability of Wilate.

**Study Design:** This study was a prospective, randomized, controlled, open-labeled, 2-arm crossover, multi-center, Phase II study. Subjects were randomly allocated to either Wilate or Humate-P for period 1. After a washout period of at least 7 days but not more than 8 weeks, subjects were switched to the other study drug for period 2.

A total of 22 subjects with inherited VWD were enrolled in the study and data from 20 subjects were evaluated for PK analysis. There were 14 females and 8 males in the study. Their age ranged from 12 to 68 years (mean 33.8 years). There were 4 subjects under the age of 18 (range: 12-17 years). The weight of the subjects ranged from 54 to 138 kilograms (mean 77.3). There were 6 subjects in Type 1 VWD, 6 in type 2A VWD, 1 in type 2B VWD, 2 in type 2M, 7 in type 3 VWD. Subjects received a bolus dose of at least 40 IU VWF:RCo/kg body weight intravenously. Blood samples for the PK evaluation were collected at baseline (within 30 minutes before injection) and at 15 and 30 minutes, and at 1, 3, 6, 12, 24, 48, and 72 hours post-injection. Approximately 15 mL of blood was collected for each PK assay and 15 mL for each VWF multimer analysis. For each subject, and in each treatment period, samples for the determination of VWF multimeric pattern were collected at pre-injection, and at 15 minutes, 1, 6, 24, and 72 hours, post injection.

Non-compartmental method was used to analyze plasma concentrations-time data using -----(b)(4)------. Pre-injection plasma concentrations were subtracted from all subsequent measurements, as they were considered to reflect the endogenous background of the subjects. In 3 subjects with undetermined baseline concentrations (VWF:RCo and/or VWF:Ag) in period 1, the respective baseline value of the second period was used. Occasional negative plasma concentrations resulting from baseline subtractions were treated as zero values. Measured batch potencies were used to derive actual doses adjusted for body weight (IU/kg). The results of the pharmacokinetic study of Wilate are summarized below.

#### **Pharmacokinetics of VWF ristocetin co-factor activity (VWF:RCo): Modified Assay**

The actual dose of Humate-P administered to patients with VWD type 3 (n = 6) was 1.4 times higher than Wilate (Table I). However, the dose-normalized  $C_{max}$  and  $AUC_{(0-infinity)}$  were

comparable between the two products. Terminal half-lives, in-vivo recovery (IVR), clearance (CL), volume of distribution at steady state (V<sub>ss</sub>) and mean residence time (MRT) were not significantly different between the two products (Table I). The 90% confidence interval (n = 20) for non-normalized dose did not meet the bioequivalence criteria of 0.8 to 1.25 (Table II). The reason for not normalizing the dose for bioequivalence assessment is not clear.

**Table I: Summary of PK results for VWF:RCo (modified) assay**

PK Parameter <sup>+</sup>	VWD Type 3 (n=6)		All VWD Types (n=20)	
	Wilate	Humate-P®	Wilate	Humate-P®
Dose	38.4 (2.9)	52.3 (3.2)	40.3 (2.7)	54.0 (3.9)
(IU/kg)	34.5-41.6	48.0-55.6	34.5-44.2	48.0-62.0
Terminal half-life	9.1 (2.6)	10.2 (2.1)	15.8 (11.0)	12.8 (3.2)
(hr)	5.7-12.9	7.2-12.5	5.7-48.5	7.2-20.0
AUC <sub>0-12</sub>	988 (300)	1428 (503)	1131 (436)	1579 (508)
(hr IU/dL)	527-1345	566-1951	527-2091	566-2815
AUC <sub>0-∞</sub>	995 (292)	1444 (518)	1235 (637)	1632 (529)
(hr IU/dL)	527-1306	559-1982	527-3363	559-2873
C <sub>max</sub>	79 (13)	105 (32)	76 (15)	108 (26)
(IU/dL)	65-102	50-144	40-102	50-159
Incremental IVR	2.07 (0.30)	2.01 (0.61)	1.89 (0.38)	1.99 (0.48)
(IU/dL per IU/kg)	1.8-2.6	1.0-2.6	1.0-2.6	1.0-2.7
CL	4.2 (1.4)	4.3 (2.3)	3.9 (1.5)	3.7 (1.5)
(mL/kg/hr)	3.0-6.6	2.4-8.8	1.2-7.1	1.8-8.8
V <sub>ss</sub>	49 (17)	55 (18)	70 (33)	62 (21)
(mL/kg)	30-67	42-90	30-159	36-111
MRT	11.9 (2.9)	13.8 (2.8)	20.6 (14.8)	17.4 (4.5)
(hr)	9.2-15.9	10.2-17.3	9.2-74.2	10.2-28.8

<sup>+</sup>Data shown are mean (SD) and range

**Age Effect:** The pediatric population consisted of 4 (12-16 years of age) out of 20 subjects. Although, the AUC was comparable between adolescents and adults (≥ 16 years), the half-life of Wilate VWF-RCo was 2-times longer in adolescents than adults (Table III). This may be an

artifact due to difficulty in estimating half-life from the terminal portion of plasma concentrations-time data. The pharmacokinetic parameters of Wilate are highly variable as well as due to small sample size of the pediatric population it is difficult to conclude if age has any impact on the pharmacokinetics of Wilate.

**Gender Effect:** There were 8 males and 12 females in this study. Although, the  $C_{max}$  was comparable between males and females, the AUC was 22% higher in males than females. The half-life was 6 hours longer in males than females (Table III).

**Table II: Comparison of treatments for VWF:RCo (modified) assay**

PK parameter (PP population, n=20)	Wilate Relative to Humate-P®		
	Point Estimate (geom. mean ratio, %)	90% Confidence Interval (CI)	p-value
Terminal Half Life	107.1	89.0-128.9	0.53
AUC <sub>0-12</sub>	70.4	60.6-81.8	<0.001
AUC <sub>0-∞</sub>	72.5	61.2-85.8	0.004
C <sub>max</sub>	71.5	64.2-79.7	<0.0001
Incremental IVR	95.9	87.0-105.7	0.46
CL	102.9	88.0-120.4	0.75
V <sub>ss</sub>	108.3	96.4-121.7	0.25
MRT	105.2	89.4-123.8	0.59

**Table III: Principal PK parameters per subgroup of Wilate - VWF:RCo (modified)**

Subpopulation	PK parameter (mean)			
	AUC <sub>0-12</sub> (hr IU/dl)	C <sub>max</sub> (IU/dL)	Terminal HL (hr)	IVR (% per IU/kg)
VWD type 1 (n=5)	1258	72	24.7 <sup>+</sup>	1.78
VWD type 2 (n=9)	1155	76	15.3	1.84
VWD type 3 (n=6)	988	79	9.1	2.07
Males (n=8)	1266	79	19.3	2.04
Females (n=12)	1041	74	13.5	1.80
< 16 yrs (n=4)	1169	65	26.7	1.55
≥ 16 yrs (n=16)	1121	79	13.1	1.98

### Pharmacokinetics of VWF ristocetin co-factor activity (VWF:RCo): Standard BCS Assay

The standard BCS assay provided lower concentrations for both Wilate and Humate-P. According to standard BCS assay, the actual dose of Humate-P administered to patients with VWD type 3 (n = 6) was 1.5 times higher than Wilate (Table IV). However, the dose-normalized  $C_{max}$  and  $AUC_{(0-\infty)}$  were comparable between the two products. Terminal half-lives, MRT, IVR, and  $V_{ss}$  were comparatively higher for Humate-P than Wilate (Table IV). The 90% confidence (n = 20) interval for non-normalized dose did not meet the bioequivalence criteria of 0.8 to 1.25 (Table V). The reason for not normalizing the dose is not clear.

**Table IV: Summary of PK results for VWF:RCo (standard BCS) assay**

PK Parameter*	VWD Type 3 (n=6)		All VWD Types (n=20)	
	Wilate	Humate-P†	Wilate**	Humate-P‡
Dose	26.4 (2.0)	39.4 (2.4)	27.7 (1.8)	40.8 (3.0)
(IU/kg)	23.7-28.5	36.2-41.9	23.7-30.3	36.2-46.8
Terminal half-life	17.1 (12.5)	28.9 (16.8)	16.1 (13.5)	19.2 (12.2)
(hr)	3.8-31.9	13.6-54.6	3.8-50.2	6.5-54.6
AUC <sub>0-11</sub>	478 (180)	713 (172)	442 (213)	726 (273)
(hr IU/dL)	231-708	444-923	151-948	276-1470
AUC <sub>inf</sub>	596 (297)	856 (246)	502 (311)	812 (314)
(hr IU/dL)	227-910	650-1348	136-1242	281-1596
$C_{max}$	35 (5)	57 (20)	35 (7)	64 (20)
(IU/dL)	30-45	24-77	21-49	24-114
Incremental IVR	1.35 (0.16)	1.45 (0.59)	1.28 (0.25)	1.55 (0.47)
(IU/dL per IU/kg)	1.1-1.6	0.6-1.9	0.8-1.7	0.6-2.6
CL	5.7 (3.2)	4.7 (1.0)	3.0 (3.0)	5.9 (2.9)
(mL/kg/hr)	3.0-10.6	3.0-6.9	2.2-21.7	2.7-14.1
$V_{ss}$	115 (52)	167 (100)	124 (46)	125 (72)
(mL/kg)	48-174	75-354	48-205	66-354
MRT	29.8 (24.0)	38.3 (25.3)	23.9 (20.3)	24.3 (17.5)
(hr)	8.0-57.0	15.9-76.8	5.0-65.4	9.1-76.8

\*Data shown are mean (SD) and range

\*\*Half-life in one type 1 subject implausibly long, summaries for HL, MRT,  $V_{ss}$ , CL, AUC<sub>inf</sub> based on 19 subjects only.

**Age Effect:** The standard BCS assay indicated that the half-life,  $C_{\max}$ , and AUC were comparable between adolescents and adults (Table VI).

**Gender Effect:** Although, the  $C_{\max}$  was comparable between males and females, the AUC was 64% higher in males than females. The half-life was 6 hours longer in males than females (Table VI).

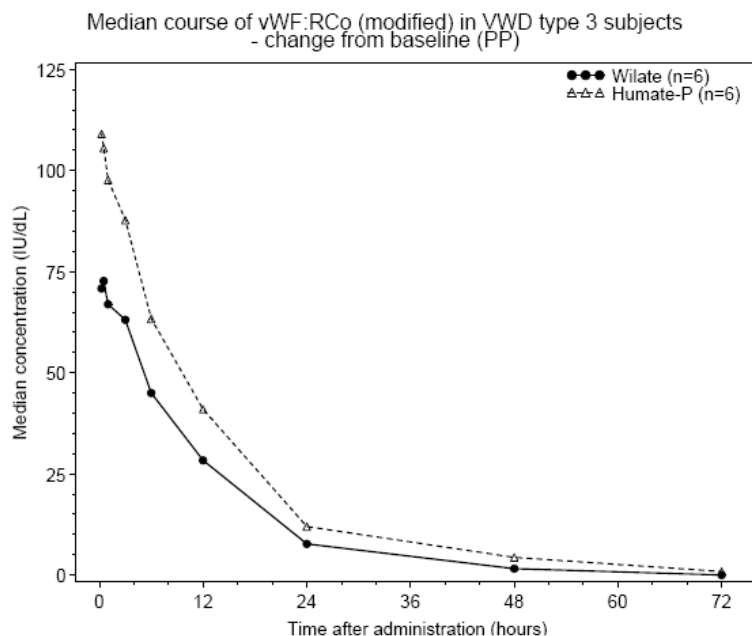
**Table V: Comparison of treatments for VWF:RCo (standard BCS) assay**

PK parameter (PP population, n=20)	Wilate Relative to Humate-P®		
	Point Estimate (geom. mean ratio, %)	90% Confidence Interval (CI)	p-value
Terminal Half Life	71.2	49.2-102.9	0.13
AUC <sub>0-12</sub>	58.0	47.9-70.3	0.0001
AUC <sub>0-∞</sub>	54.9	44.5-67.7	0.0001
C <sub>max</sub>	57.3	50.5-65.0	<0.0001
Incremental IVR	84.4	75.1-94.8	0.02
CL	124.3	101.4-152.4	0.08
V <sub>ss</sub>	104.1	84.4-128.4	0.74
MRT	83.7	62.1-112.8	0.32

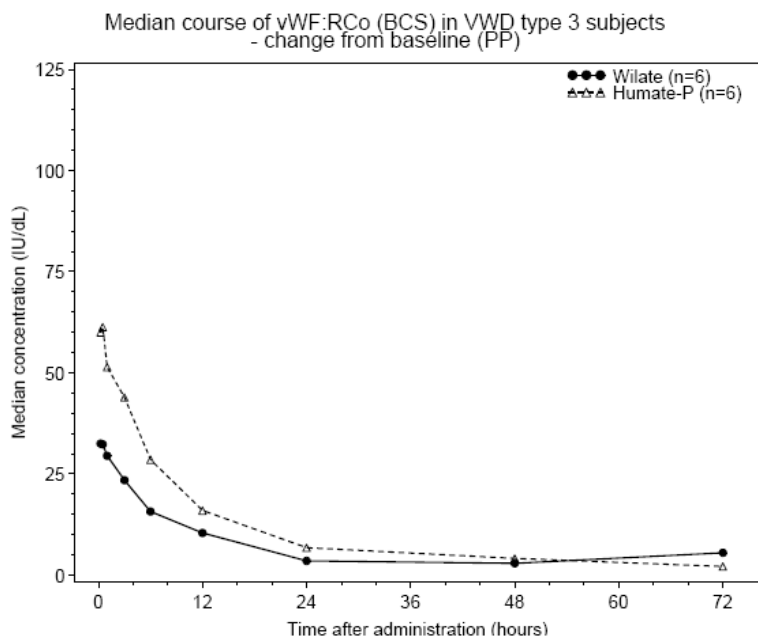
**Table VI: Principal PK parameters per subgroup of Wilate - VWF:RCo (standard BCS)**

Subpopulation	PK parameter (mean)			
	AUC <sub>0-12</sub> (hr IU/dl)	C <sub>max</sub> (IU/dL)	Terminal HL (hr)	IVR (% per IU/kg)
VWD type 1 (n=5)	570	32	26.8 <sup>+</sup>	1.15
VWD type 2 (n=9)	348	37	10.7	1.31
VWD type 3 (n=6)	478	35	17.1	1.35
Males (n=8)	578	34	20.1 <sup>+</sup>	1.29
Females (n=12)	352	36	13.8	1.27
< 16 yrs (n=4)	429	31	16.8	1.06
≥ 16 yrs (n=16)	446	37	15.9 <sup>+</sup>	1.33

**Figure 1: Median VWF:RCo (modified) profiles with VWD type 3**



**Figure 2: Median VWF:RCo (standard BCS) profiles with VWD type 3**



#### **Difference in PK by the two assay methods:**

Between the two assay methods (modified and standard BCS), the pharmacokinetic parameters are different. The mean  $C_{max}$  and  $AUC_{(0-\infty)}$  of Wilate ( $n = 20$ ) are 2 and 2.5-fold higher (without dose adjustment) by the modified BCS assay than standard BCS assay. When accounted for all 20 subjects, the mean half-life appears to be comparable by the two analytical

methods but the half-life of Wilate in patients with VWD type 3 is almost 50% shorter by modified assay than the standard BCS assay. From Figure II, it is apparent that it is difficult to calculate half-life with accuracy for Wilate from the flat terminal part of concentrations-time curve. In short, there is uncertainty about the PK parameters estimated from standard BCS assay method.

### **Pharmacokinetics of VWF antigen (VWF:Ag)**

The actual dose of Humate-P administered to patients with VWD type 3 ( $n = 6$ ) was 1.3 times higher than Wilate (Table VII). The dose-normalized  $C_{\max}$  and  $AUC_{(0-\infty)}$  were comparable between the two products. Terminal half-lives, IVR, CL, Vss and MRT were not significantly different between the two products (Table VII). The 90% confidence interval ( $n = 20$ ) for non-normalized dose did not meet the bioequivalence criteria of 0.8 to 1.25 (Table VIII).

**Table VII: Summary of PK results for VWF:Ag assay**

PK Parameter <sup>†</sup>	VWD Type 3 (n=6)		All VWD Types (n=20)	
	Wilate	Humate-P®	Wilate	Humate-P®
<b>Dose</b>	40.7 (3.0)	54.6 (3.3)	42.7 (2.8)	56.4 (4.1)
(IU/kg)	36.6-44.0	50.1-58.0	36.5-46.7	50.1-64.7
<b>Terminal half-life</b>	16.1 (7.0)	13.1 (3.1)	18.5 (7.0)	18.5 (9.5)
(hr)	9.1-27.6	10.0-17.9	8.2-29.7	10.0-51.0
<b>AUC<sub>0-12</sub></b>	1328 (380)	1686 (379)	1434 (440)	1945 (580)
(hr IU/dL)	808-1752	1210-2225	808-2705	1210-3244
<b>AUC<sub>0-∞</sub></b>	1485 (379)	1831 (536)	1600 (538)	2152 (727)
(hr IU/dL)	970-2047	1091-2593	826-3252	1091-3822
<b>C<sub>max</sub></b>	91 (12)	113 (23)	86 (13)	113 (20)
(IU/dL)	76-110	74-138	61-111	74-151
<b>Incremental IVR</b>	2.24 (0.29)	2.07 (0.42)	2.04 (0.33)	2.02 (0.38)
(IU/dL per IU/kg)	1.9-2.6	1.4-2.5	1.4-2.6	1.4-2.7
<b>CL</b>	2.9 (0.7)	3.2 (1.0)	2.9 (0.9)	2.9 (0.9)
(mL/kg/hr)	1.8-3.8	1.9-4.7	1.3-5.2	1.4-4.7
<b>V<sub>ss</sub></b>	58 (22)	54 (10)	68 (20)	65 (25)
(mL/kg)	31-84	44-65	31-109	44-150
<b>MRT</b>	20.7 (8.1)	17.7 (4.4)	25.0 (9.0)	24.3 (10.3)
(hr)	12.6-34.7	13.9-24.0	11.8-39.8	13.9-50.6

<sup>†</sup>Data shown are mean (SD) and range



**Table VIII: Comparison of treatments for VWF:Ag assay**

PK parameter (PP population, n=20)	Wilate Relative to Humate-P®		
	Point Estimate (geom. mean ratio, %)	90% Confidence Interval (CI)	p-value
Terminal Half Life	101.8	86.9-119.3	0.85
AUC <sub>all</sub>	73.6	66.0-82.0	0.0001
AUC <sub>inf</sub>	74.7	66.2-84.2	0.0005
C <sub>max</sub>	76.7	71.6-82.1	<0.0001
Incremental IVR	101.3	95.6-107.4	0.70
CL	101.4	90.4-113.7	0.84
V <sub>ss</sub>	105.2	95.8-115.6	0.36
MRT	103.8	89.7-120.1	0.66

**Table IX: Principal PK parameters per subgroup of Wilate - VWF:Ag**

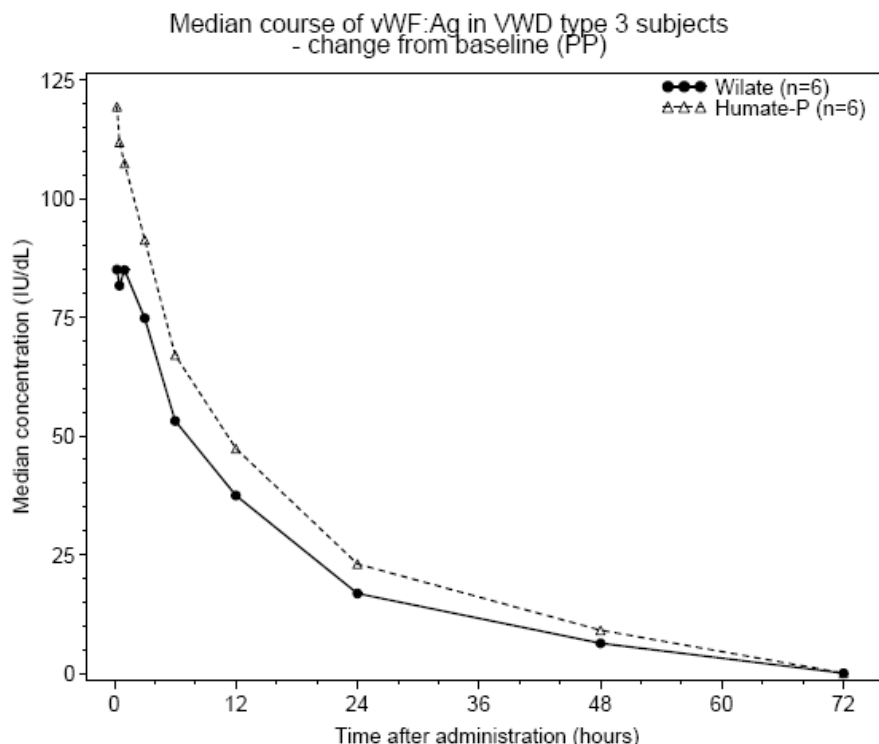
Subpopulation	PK parameter (mean)			
	AUC <sub>all</sub> (hr IU/dl)	C <sub>max</sub> (IU/dL)	Terminal HL (hr)	IVR (% per IU/kg)
VWD type 1 (n=5)	1386	80	18.5	1.90
VWD type 2 (n=9)	1531	87	20.1	1.97
VWD type 3 (n=6)	1328	91	16.1	2.24
Males (n=8)	1622	93	19.9	2.26
Females (n=12)	1308	82	17.6	1.89
< 16 yrs (n=4)	1304	79	23.8	1.79
≥ 16 yrs (n=16)	1466	88	17.2	2.10

**Age Effect:** Although, the AUC was comparable between adolescents and adults, the half-life of VWF:Ag was 6 hours longer in adolescents than adults.

**Gender Effect:** Although, the C<sub>max</sub> was comparable between males and females, the AUC was

24% higher in males than females. The half-life was comparable between males and females.

**Figure 3: Median VWF:Ag profiles with VWD type 3**



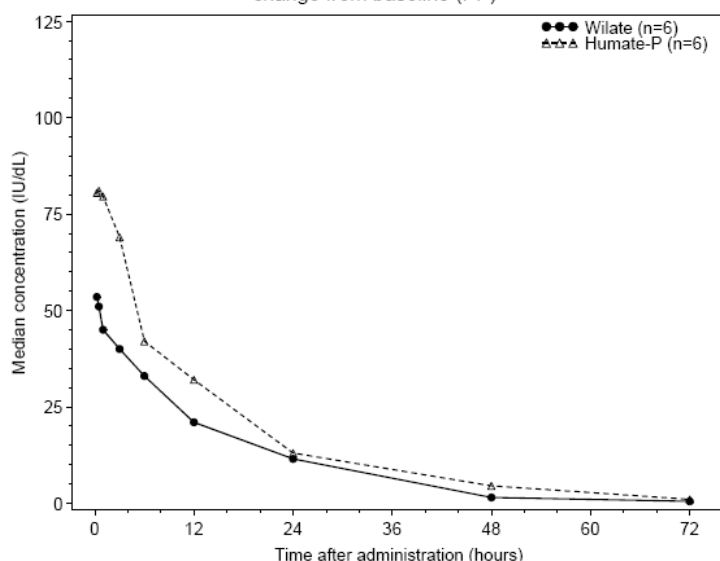
#### Pharmacokinetics of VWF collagen binding activity (VWF:CBA)

The actual dose of Humate-P administered to patients with VWD type 3 ( $n = 6$ ) was 1.15 times higher than Wilate (Table X). The dose-normalized  $C_{\max}$  and  $AUC_{(0-\infty)}$  were not comparable between the two products. After normalizing the dose, the  $C_{\max}$  and  $AUC_{(0-\infty)}$  were at least 1.4 times higher for Humate-P than Wilate. Both half-life and MRT were longer by 5 hours for Humate-P than Wilate. The 90% confidence interval ( $n = 20$ ) for non-normalized dose did not meet the bioequivalence criteria of 0.8 to 1.25 (Table XI).

**Age Effect:** Although, the AUC was 20% less in adolescents than adults, the half-life of Wilate was 12 hours longer in adolescents than adults (Table XII).

**Gender Effect:** The  $C_{\max}$ , AUC, and half-life were comparable between males and females (Table XII).

**Figure 4: Median VWF:CBA profiles with VWD type 3**  
 Median course of vWF:CBA in VWD type 3 subjects  
 - change from baseline (PP)



**Table X: Summary of PK results for VWF:CB assay**

PK Parameter*	VWD Type 3 (n=6)		All VWD Types (n=20)	
	Wilate	Humate-P®	Wilate	Humate-P®
Dose	37.4 (2.8)	43.0 (2.6)	39.3 (2.6)	44.4 (3.2)
(IU/kg)	33.7-40.6	39.4-45.7	33.6-43.0	39.4-51.0
Terminal half-life	10.9 (5.7)	15.2 (9.6)	16.2 (7.7)	18.2 (14.4)
(hr)	2.2-17.1	7.9-33.2	2.2-34.4	7.8-67.0
AUCall	742 (399)	1203 (352)	784 (296)	1503 (1289)
(hr IU/dL)	15-1098	863-1809	15-1326	745-6863
AUCinf	763 (411)	1268 (359)	859 (348)	1955 (2790)
(hr IU/dL)	18-1101	893-1914	18-1617	767-13729
Cmax	52 (27)	86 (19)	52 (19)	97 (28)
(IU/dL)	6-85	62-119	6-85	62-168
Incremental IVR	1.44 (0.79)	2.03 (0.55)	1.35 (0.55)	2.20 (0.63)
(IU/dL per IU/kg)	0.1-2.5	1.4-3.0	0.1-2.5	1.4-3.6
CL	41.3 (90.9)	3.6 (0.9)	16.0 (49.7)	3.4 (1.2)
(mL/kg/hr)	3.4-226.8	2.1-4.5	2.4-226.8	0.3-6.0
Vss	177 (261)	73 (52)	151 (176)	68 (35)
(mL/kg)	47-709	44-178	47-709	34-178
MRT	15.0 (7.4)	20.3 (12.1)	22.4 (11.2)	24.7 (20.7)
(hr)	3.1-24.2	12.0-43.5	3.1-46.4	11.1-100.1

\*Data shown are mean (SD) and range

**Table XI: Comparison of treatments for VWF:CB assay**

PK parameter (PP population, n=20)	Wilate Relative to Humate-P®		
	Point Estimate (geom. mean ratio, %)	90% Confidence Interval (CI)	p-value
Terminal Half Life	93.2	71.5-121.6	0.65
AUCall	49.2	34.0-71.2	0.004
AUCinf	48.3	33.1-70.3	0.004
Cmax	48.7	38.2-62.2	<0.0001
Incremental IVR	55.1	42.7-71.1	0.0007
CL	183.4	125.1-268.8	0.013
Vss	176.1	129.5-239.3	0.005
MRT	96.0	73.6-125.3	0.80

**Table XII: Principal PK parameters per subgroup of Wilate – VWF:CB**

Subpopulation	PK parameter (mean)			
	AUCall (hr IU/dl)	Cmax (IU/dL)	Terminal HL (hr)	IVR (% per IU/kg)
VWD type 1 (n=5)	716	50.2	16.8	1.30
VWD type 2 (n=9)	850	53.1	19.4	1.31
VWD type 3 (n=6)	742	52.3	10.9	1.44
Males (n=8)	772	46.4	16.3	1.26
Females (n=12)	792	56.0	16.1	1.40
< 16 yrs (n=4)	651	43.3	25.3	1.05
≥ 16 yrs (n=16)	818	54.4	13.9	1.42

### Pharmacokinetics of Factor VIII Coagulant Activity (FVIII:C): One Stage Assay

Median plasma concentrations of FVIII:C (---(b)(4)---) for Wilate was higher than Humate-P for the first 12 hours and then declined with much faster rate than Humate-P (Figure 5). The actual dose of Wilate administered to patients with VWD type 3 was 2.3 times higher than Humate-P (Table XIII). The dose-normalized  $C_{max}$  and  $AUC_{(0-\infty)}$  for patients with VWD type 3 were not comparable between the two products. After normalizing the dose, the  $C_{max}$  and  $AUC_{(0-\infty)}$  were about 60% lower for Wilate than Humate-P. Half-life was longer by 5 hours for Humate-P than Wilate. The 90% confidence interval for AUC (non-normalized dose) was within the limits of 0.8 to 1.25 (Table XIV) but  $C_{max}$  was outside this limit.

**Age Effect:** Although, the AUC of Wilate was 30% less in adolescents than adults, the  $C_{max}$  and half-life of Wilate were comparable between the two groups (Table XV).

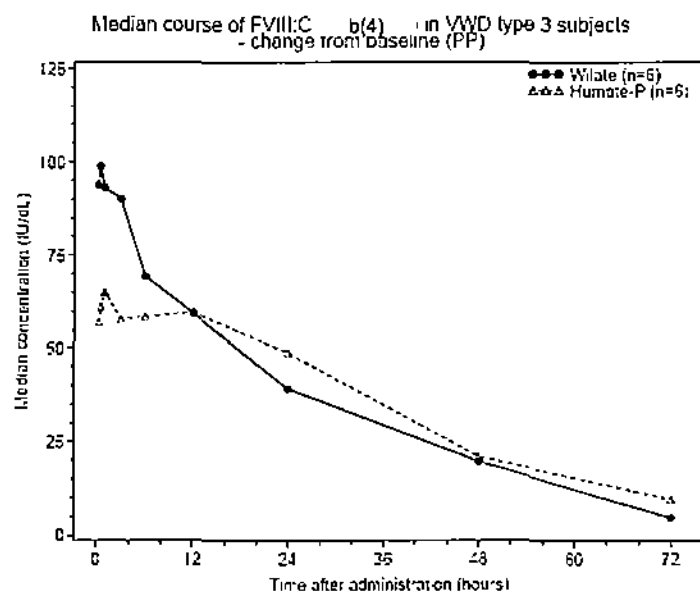
**Gender Effect:** The AUC was 34% higher in males than females and half-life was 5 hours shorter in males than females (Table XV).

Table XIII: Summary of PK results for FVIII:C (---(b)(4)---) assay

PK Parameter*	VWD Type 3 (n=6)		All VWD Types (n=20)	
	Wilate	Humate-P	Wilate	Humate-P
Dose	48.6 (3.6)	21.0 (1.3)	51.0 (3.4)	21.7 (1.6)
(IU/kg)	43.7-52.7	19.3-22.3	45.7-55.9	19.3-24.9
Terminal half-life	18.2 (4.1)	23.3 (11.4)	21.9 (10.5)	26.8 (14.4)
(hr)	11.6-23.6	13.2-43.2	12.3-55.3	9.4-65.4
AUC <sub>0-12</sub>	2435 (853)	2634 (637)	1882 (766)	1842 (735)
(hr IU/dL)	1899-3913	1734-3558	1127-3913	102-3558
AUC <sub>0-24</sub>	2823 (915)	2967 (719)	2254 (779)	2251 (922)
(hr IU/dL)	1939-3930	1895-3815	1140-3980	419-3815
$C_{max}$	101 (13)	71 (17)	109 (29)	61 (15)
(IU/dL)	89-116	48-100	76-200	33-100
Incremental IVR	2.09 (0.29)	3.39 (0.81)	2.12 (0.53)	2.81 (0.72)
(IU/dL per IU/kg)	1.7-2.4	2.5-4.8	1.4-3.7	1.5-4.6
CL	1.8 (0.6)	0.8 (0.2)	2.5 (0.9)	1.3 (1.0)
(mL/kg/hr)	1.3-2.6	0.5-1.2	1.3-4.5	0.5-4.8
$V_{ss}$	49 (14)	25 (8)	71 (39)	42 (22)
(mL/kg)	29-66	15-37	29-150	15-97
30RT	26.2 (4.0)	34.7 (13.1)	31.3 (14.6)	32.1 (18.8)
(hr)	22.5-32.0	25.6-57.5	12.6-65.1	15.3-87.5

\*Data shown are mean (SD) and range

**Figure 5: Median FVIII:C b(4) profiles with VWD type 3**



**Table XIV: Comparison of treatments for FVIII:C b(4) assay**

PK parameter (PP population, n=20)	Wilate Relative to Humate-P		
	Point Estimate (geom. mean ratio, %)	90% Confidence Interval (CI)	p-value
Terminal Half Life	85.1	67.8-106.7	0.23
AUC <sub>0-11</sub>	113.2	99.3-128.9	0.12
AUC <sub>inf</sub>	107.3	90.9-126.7	0.47
C <sub>max</sub>	179.3	164.5-195.5	<0.0001
Incremental IVR	76.3	70.3-82.9	<0.0001
CL	219.0	186.5-257.2	<0.0001
V <sub>ss</sub>	177.1	157.4-199.2	<0.0001
MRT	80.8	66.7-98.0	0.07

Table XV: Principal PK parameters per subgroup of Wilate – FVIII:C --(b)(4)--

Subpopulation	PK parameter (mean)			
	AUC <sub>0-12</sub> (hr IU/dL)	C <sub>max</sub> (IU/dL)	Terminal HL (hr)	IVR (% per IU/kg)
VWD type 1 (n=5)	1661	109	17.5	2.34
VWD type 2 (n=9)	1725	114	26.8	2.36
VWD type 3 (n=6)	2635	101	18.2	2.09
Males (n=8)	2338	106	18.8	2.17
Females (n=12)	1745	111	24.0	2.11
< 16 yrs (n=4)	1482	101	23.4	1.92
≥ 16 yrs (n=16)	2108	111	21.5	2.19

#### Factor VIII Coagulant Activity (FVIII:C): Chromogenic assay

Median plasma concentrations of FVIII:C --(b)(4)-- for Wilate was higher than Humate-P for the first 12 hours and then declined with much faster rate than Humate-P (Figure 6). The actual dose of Humate-P administered to patients with VWD type 3 was 50% lower than Wilate (Table XVI). The dose-normalized C<sub>max</sub> and AUC<sub>(0-infinity)</sub> were not comparable between the two products. After normalizing the dose, the C<sub>max</sub> and AUC<sub>(0-infinity)</sub> were about 50% lower for Wilate than Humate-P. Half-life was longer by 5 hours for Humate-P than Wilate. The 90% confidence interval for AUC (non-normalized dose) was within the limits of 0.8 to 1.25 (Table XVII) but C<sub>max</sub> was outside this limit.

**Age Effect:** Although, the AUC and C<sub>max</sub> of Wilate was 25% less in adolescents than adults but the half-life was comparable between the two groups (Table XVII).

**Gender Effect:** The AUC of wilate was 34% higher in males than females but C<sub>max</sub> and half-life were comparable between the two groups (Table XVII).

Figure 6: Median FVIII:C (chromogenic) profiles with VWD type 3

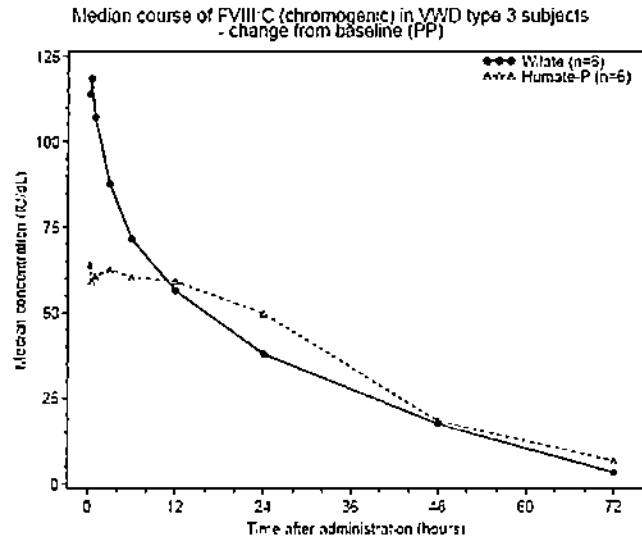


Table XVI: Summary of PK results for FVIII:C (chromogenic) assay

PK Parameter <sup>***</sup>	VWD Type 3 (n=6)		All VWD Types (n=20)	
	Wdate	Humate-P <sup>†</sup>	Wdate <sup>***</sup>	Humate-P <sup>†</sup>
Dose	48.7 (3.6)	24.8 (1.5)	51.1 (3.4)	25.6 (1.9)
(IU/kg)	43.8-52.8	22.7-26.5	45.8-56.0	22.7-29.4
Terminal half-life	16.1 (3.1)	20.5 (7.6)	15.6 (6.9)	24.6 (11.8)
(hr)	11.8-26.1	10.7-39.8	10.9-34.7	10.7-63.9
AUC <sub>0-11</sub>	2545 (776)	2449 (497)	1598 (756)	1773 (662)
(hr IU/dL)	1844-3513	1872-3063	440-3513	639-3063
AUC <sub>inf</sub>	2670 (854)	2692 (610)	2290 (1043)	2103 (817)
(hr IU/dL)	1874-3655	2018-3679	464-4424	663-3679
C <sub>max</sub>	120 (23)	67 (10)	112 (23)	61 (10)
(IU/dL)	91-148	50-77	59-148	42-77
Incremental IVR	2.47 (0.47)	2.69 (0.34)	2.19 (0.48)	2.38 (0.41)
(IU/dL per IU/kg)	2.0-3.0	2.2-3.1	1.1-3.0	1.7-3.1
CL	2.0 (0.6)	1.0 (0.2)	2.9 (2.1)	1.5 (0.8)
(mL/kg/hr)	1.4-2.8	0.6-1.3	1.2-11.0	0.6-4.0
V <sub>ss</sub>	24 (10)	38 (8)	72 (58)	47 (20)
(mL/kg)	22-57	21-39	52-190	21-164
MRT	23.0 (3.7)	20.3 (8.5)	28.4 (11.1)	35.7 (16.7)
(hr)	13.0-37.7	21.9-45.7	17.2-61.6	20.2-50.6

<sup>†</sup>Data shown are mean (SD) and range

<sup>\*\*\*</sup>Half-life in one type 2 subject [66] implausibly long, summaries for HL, MRT, V<sub>ss</sub>, CL, AUC<sub>inf</sub> based on 19 subjects only.



**Table XVII: Comparison of treatments for FVIII:C (chromogenic) assay**

PK parameter (PP population, n=20)	Wilate Relative to Humate-P®		
	Point Estimate (geom. mean ratio, %)	90% Confidence Interval (CI)	p-value
<b>Terminal Half Life</b>	84.7	71.6-100.2	0.10
<b>AUC<sub>0-12</sub></b>	111.0	98.1-125.7	0.16
<b>AUC<sub>0-∞</sub></b>	105.9	90.0-124.7	0.55
<b>C<sub>max</sub></b>	182.5	171.1-194.6	<0.0001
<b>Incremental IVR</b>	91.3	86.4-96.5	0.01
<b>CL</b>	188.0	160.2-220.6	<0.0001
<b>V<sub>ss</sub></b>	156.3	142.2-171.9	<0.0001
<b>MRT</b>	83.1	71.5-96.7	0.048

**Table XVIII: Principal PK parameters per subgroup of Wilate - FVIII:C (chromogenic)**

Subpopulation	PK parameter (mean)			
	AUC <sub>0-12</sub> (hr IU/dl)	C <sub>max</sub> (IU/dL)	Terminal HL (hr)	IVR (% per IU/kg)
<b>VWD type 1 (n=5)</b>	1432	94	17.5	1.86
<b>VWD type 2 (n=9)</b>	1947	115	23.6 <sup>+</sup>	2.19
<b>VWD type 3 (n=6)</b>	2545	120	16.1	2.47
<b>Males (n=8)</b>	2356	118	18.0	2.40
<b>Females (n=12)</b>	1759	108	20.9 <sup>+</sup>	2.05
<b>&lt; 16 yrs (n=4)</b>	1548	89	21.8 <sup>+</sup>	1.67
<b>≥ 16 yrs (n=16)</b>	2110	117	19.2	2.32

## CONCLUSIONS/COMMENTS

1. The modified analytical method for the measurement of VWF:RCo appears to be more sensitive than the standard BCS analytical method. The plasma concentrations-time data appear to follow a pattern (decline with time) with the modified analytical method than the BCS analytical method. This may be because at lower concentrations of VWF:RCo, the standard BCS is not as sensitive as the modified method. It also appears that the concentrations measured by BCS are at least by 1.75 times lower (calculated based on mean AUC and per mg dose) than the concentrations measured by the modified BCS method. The  $C_{\max}$  is 1.5 times lower (calculated based on mean  $C_{\max}$  and per mg dose) by BCS analytical method than by the modified BCS method.
2. Between the two assay methods (modified and standard BCS), the pharmacokinetic parameters are different. The mean  $C_{\max}$  and  $AUC_{(0-\infty)}$  (without dose adjustment) of Wilate ( $n = 20$ ) are 2 and 2.5-fold higher by the modified assay than standard BCS assay. When accounted for all 20 subjects, the mean half-life appears to be comparable by the two analytical methods but the half-life of Wilate in patients with VWD type 3 is almost 50% shorter by modified assay than the standard BCS assay. From Figure II, it is apparent that it is difficult to calculate half-life with accuracy for Wilate from the flat terminal part of concentrations-time curve using standard BCS method. In short, there is uncertainty about the PK parameters estimated from standard BCS assay method.
3. Considering that most of the laboratories use the standard method, the FDA should consider either changing the analytical method for the measurement of VWF:RCo (from standard BCS to modified method) or apply a correction factor. A correction factor however, may not be uniform across all concentrations of VWF:RCo. For example, two or three different correction factors may be needed for low, medium, and high concentrations of VWF:RCo. In short, a pragmatic solution should be sought to reconcile the differences in plasma concentrations of Wilate introduced by the two analytical methods. In the clinical setting the manual method will be used to monitor treatment with Wilate and should be sufficiently sensitive for this purpose.