

Biochemical quality of the pharmaceutically licensed plasma Octaplas® after implementation of a novel prion protein (PrP^{Sc}) removal technology

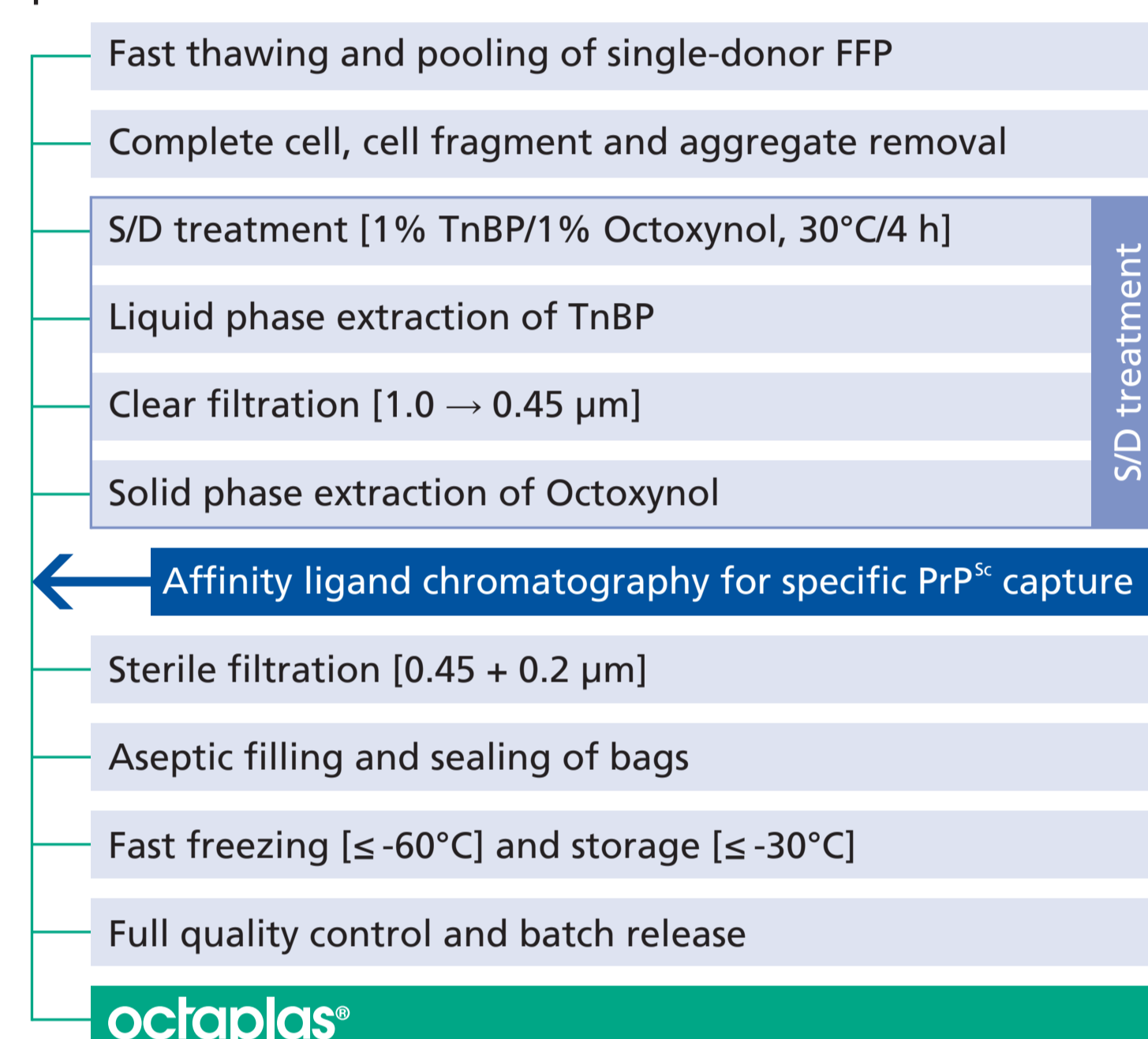
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Introduction

The implementation of novel technologies into manufacturing processes may have an impact on the quality of plasma-derived products. A new chromatographic step for the selective binding of PrP^{Sc} to an affinity ligand, developed and optimised for PrP^{Sc} capture and attached to synthetic resin particles [developed by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., US)], was implemented into the manufacturing process of the solvent/detergent (S/D) treated biopharmaceutical plasma Octaplas® (for flow-chart of the manufacturing process, see **Figure 1**). The column was incorporated after the complete cell removal and S/D treatment, representing a stage at which cells and debris that might harbour PrP^{Sc} have been fully removed (i.e. clear, liquid matrix).

Figure 1: Flow-chart of the Octaplas® manufacturing process



Affinity ligand chromatography for specific prion protein (PrP^{Sc}) capture was implemented after cell filtration and S/D treatment.

The aim of these studies was not only to evaluate the technical performance of the incorporated chromatographic step, but also to demonstrate that the quality of Octaplas® is not impaired by the introduction of this novel technology at large-scale routine manufacturing.

Materials & Methods

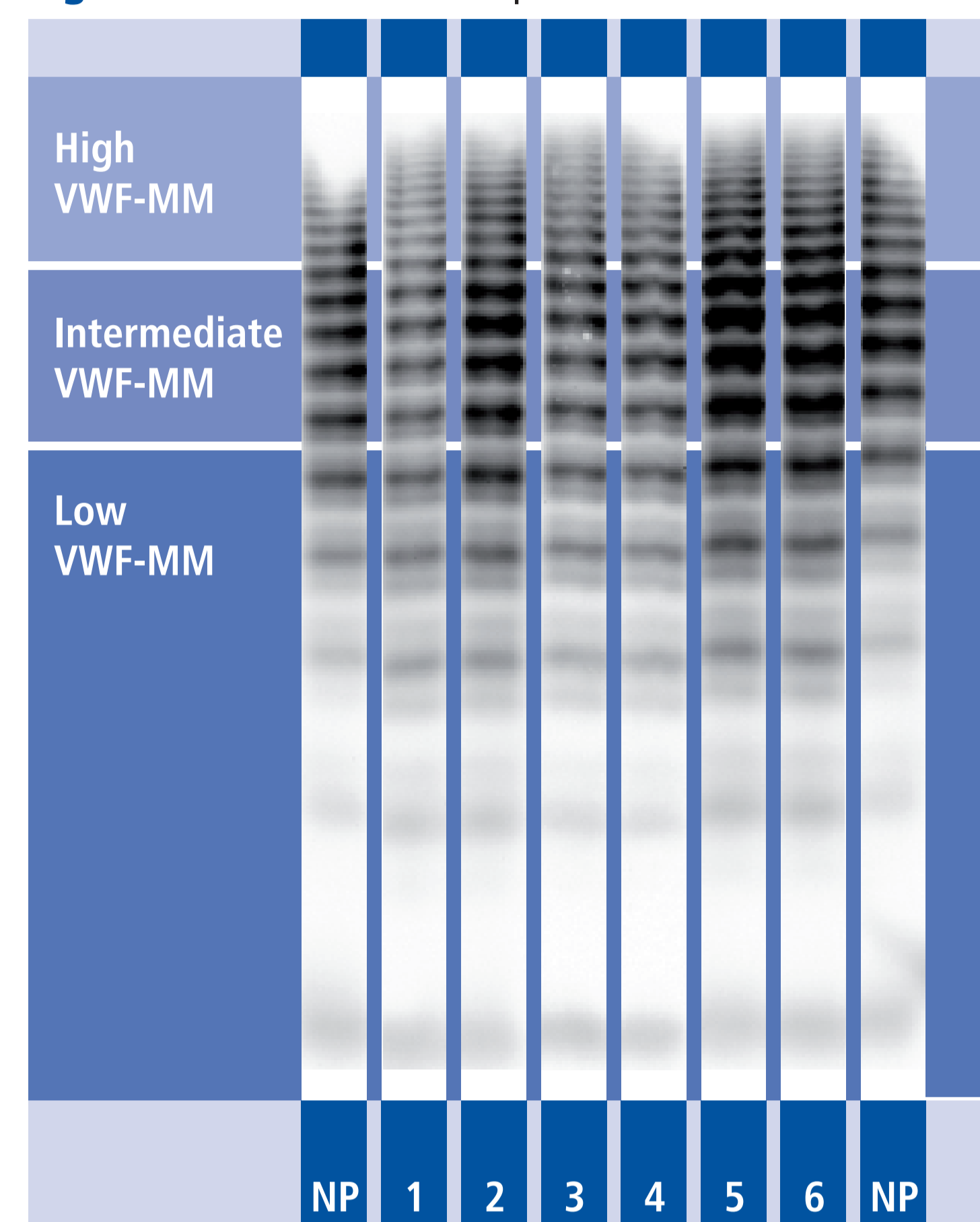
Three consecutive scale-up batches of Octaplas® with implemented affinity ligand chromatography for specific PrP^{Sc} capture [labelled as Octaplas® LG (ligand gel) in the study] were manufactured by Octapharma PPGmbH, Vienna, Austria. Final containers of normal Octaplas® produced without the additional prion protein removal step were used as control samples.

The biochemical quality of Octaplas® LG was determined directly after manufacturing, as well as after one year storage at ≤ -30°C and -18°C ± 2°C. All plasma samples were tested on the current product release parameters (i.e. total protein, fibrinogen, coagulation factors V, VIII and XI (FV, FVIII and FXI), activated and non-activated partial thromboplastin time (aPTT and NAPTT), anti-hepatitis A virus (anti-HAV) IgG, anti-parvovirus B19 (anti-B19) IgG, pH value, osmolality, sodium, potassium, calcium, citrate, phosphate, glycine, TnBP, and Octoxynol), using the validated routine test methods. Additional parameters included global coagulation parameters [prothrombin time (PT), reptilase time (RT) and thrombin time (TT)], thrombin generation assay (TGA), fibrinogen levels, the activities of coagulation factors [factors II, VII, IX, X, XII and XIII (FII, FVII, FIX, FX, FXII and FXIII)] and protease inhibitors [antithrombin III (ATIII), heparin co-factor II (HCII), protein C (PC), protein S (PS), plasmin inhibitor (PI, also known as α₂-antiplasmin), α₁-antitrypsin (A1AT) and C1-esterase inhibitor (C1-INH)], as well as ADAMTS13 (VWF cleaving protease) activity and antigen levels. Finally, markers of activated coagulation and fibrinolysis [activated factor VII (FVIIa), thrombin-antithrombin complex (TAT), prothrombin fragments I+II (F1+2) and D-dimer] were measured and von Willebrand factor (VWF) multimeric analyses were performed.

Results

From the manufacturing crew it was reported that the performance of the chromatography step was very good in the industrial set-up developed by Octapharma. The flow-rate, ensuring the correct contact time between the plasma matrix and affinity ligand gel, could be well controlled. The integrity of the column was fully maintained, i.e. no channelling, and there was no major prolongation of the total production time.

Figure 2: VWF multimeric pattern



Low (1-5), intermediate (6-10) and high (> 11) molecular weight VWF multimers (VWF-MM) are indicated. NP, Normal Plasma Reference Standard. Results for three consecutive batches of Octaplas® (1,3,5) and Octaplas® LG (2,4,6) are presented.

After thawing, all three Octaplas® LG batches were clear and free of solid and gelatinous particles, and pH and osmolality remained within the approved specification for Octaplas® (**Table 1**). No prolongation of the PT, aPTT and NAPTT was observed, indicating no relevant depletion of coagulation factors required for either the extrinsic or the intrinsic pathway of coagulation. In addition, there was no prolongation of the TT and RT by decreased or dysfunctional fibrinogen observed (**Table 2**). The thrombin generation potential was assessed and compared, reflecting a composite effect of the multiple factors determining coagulation capacity. Generated thrombin concentrations (mean 427-438 nM, **Figure 3**) were within the reference ranges indicated in the lot-specific batch table of the Technoclone test kit. All coagulation factor activities were higher than 0.5 IU/ml, fibrinogen levels varied between 2.6 and 2.8 mg/ml, i.e. within the normal ranges for plasma (**Table 2**). Activities of ATIII, HCII, PC, A1AT and C1-INH were within the normal ranges for single-donor fresh-frozen plasma (FFP) (**Table 2**). PS and PI activities were within the Ph.Eur.-recommended levels for S/D plasma (i.e. Ph.Eur. 6.1, 07/2008:1646; valid as of July 2008), and showed no significant differences to the activities measured in the current version of Octaplas® (**Table 2**). In addition, ADAMTS13, which is important for the physiological size and function of VWF multimers, was measured. There were no significant differences in ADAMTS13 activity and antigen levels between the two Octaplas® products (**Table 2**). Finally, indicators of coagulation activation and fibrinolysis were tested and VWF multimeric analyses were performed. FVIIa levels ranged between 81-90 mIU/ml, indicating that there was no activation of FVII caused by the implemented ligand chromatography step. TAT levels (result of low level thrombin generation and subsequent inhibition by antithrombin), F1+2 levels (indicator of traces of prothrombin activation) and D-dimer levels (released by plasmin action towards formed fibrin) were within the normal ranges (**Table 2**). All plasma samples showed VWF multimeric patterns comparable to that of normal plasma. There were no ultra-large molecular weight forms of VWF multimers observed in any of the plasma samples tested (**Figure 2**). Furthermore, the triplet structure of VWF was found to be normal, i.e. not subjected to proteolytic cleavage.

Extensive stability studies showed no significant changes in the plasma quality of Octaplas® LG with the implemented prion protein removal step after one year storage at both ≤ -30°C and -18°C ± 2°C. All coagulation factor and protease inhibitor activities remained within the normal ranges for S/D plasma (mean activities in IU/ml and in percent relative to the activities at time point 0 are presented after one year storage at ≤ -30°C; **Figure 4/1** and **4/2**, respectively). The TGA showed stable results, within the assay variances, for Octaplas® LG during one year storage. No activation of parameters of blood coagulation and fibrinolysis occurred. No impact on the VWF multimeric pattern was observed when compared with normal plasma. The stability studies continue.

Conclusion

- The incorporation of this novel affinity ligand chromatography for selective PrP^{Sc} capture was technically feasible and the performance of the column was at a full Good Manufacturing Practice (GMP) level.
- The biochemical studies showed that Octaplas® produced with and without the new column demonstrate an identical medicinal quality. In addition, extensive

Table 1: Standard Octaplas® quality control (QC) release parameters

Parameters	Ph.Eur. 5.0 01/2005:1646	Octaplas® Final Product Specification	Octaplas® Mean ± St.dev. (n=3)	Octaplas® LG Mean ± St.dev. (n=3)
Total protein [mg/ml]	≥ 45	45-70	62 ± 1	61 ± 2
Fibrinogen [mg/ml]	not specified	1.5-4.0	2.7 ± 0.1	2.6 ± 0.1
Factor V [IU/ml]	≥ 0.5	≥ 0.5	0.8 ± 0.1	0.8 ± 0.1
Factor VIII [IU/ml]	≥ 0.5	≥ 0.5	0.8 ± 0.2	0.7 ± 0.2
Factor XI [IU/ml]	≥ 0.5	≥ 0.5	0.8 ± 0.1	0.8 ± 0.0
aPTT [sec]	not specified	23-40	29 ± 1	29 ± 1
NAPTT [sec]	≥ 150	≥ 150	281 ± 25	289 ± 81
Anti-HAV IgG [IU/ml]	≥ 2 (≥ 1)*	≥ 1	1.7 ± 0.6	1.7 ± 0.6
Anti-B19 IgG [IU/ml]	not specified	≥ 20	34 ± 2	32 ± 2
Osmolality [mosmol/kg]	≥ 240	320-420	359 ± 5	357 ± 7
Sodium [mmol/l]	≤ 200	≤ 200	159 ± 8	159 ± 5
Potassium [mmol/l]	≤ 5.0	≤ 5.0	3.6 ± 0.2	3.7 ± 0.1
Calcium [mmol/l]	≤ 5.0	≤ 5.0	2.0 ± 0.1	2.0 ± 0.0
Citrate [mmol/l]	≤ 25	15-25	19 ± 2	20 ± 2
Phosphate [mmol/l]	not specified	2.0-7.5	5.3 ± 0.9	5.2 ± 0.9
Glycine [mg/ml]	not specified	4.0-6.0	5.1 ± 0.1	5.1 ± 0.1
TNBP [µg/ml]	< 2	≤ 2.0	Comply (< 0.5)	Comply (< 0.5)
Octoxynol [µg/ml]	< 5	≤ 5.0	Comply (< 1.0)	Comply (< 0.9)

Octaplas® produced with ligand chromatography for specific PrP^{Sc} capture was labelled as Octaplas® LG.

St. dev., standard deviation

*In the process of being changed to 1 IU/ml.

Table 2: Global parameters of blood coagulation and inhibition in Octaplas®

Parameters	Reference Range [1]	Octaplas® Mean ± St.dev. (n=3)	Octaplas® LG Mean ± St.dev. (n=3)
PT [sec]	10-14	10.5 ± 0.5	10.4 ± 0.6
RT [sec]	16-24	20.3 ± 2.7	19.6 ± 1.0
TT [sec]	14-20	14.7 ± 1.4	14.5 ± 1.0
Factor II [IU/ml]	0.65-1.54	0.98 ± 0.05	0.94 ± 0.03
Factor VII [IU/ml]	0.62-1.65	0.93 ± 0.11	0.90 ± 0.06
Factor IX [IU/ml]	0.45-1.48	0.84 ± 0.09	0.83 ± 0.07
Factor X [IU/ml]	0.68-1.48	1.03 ± 0.11	1.01 ± 0.10
Factor XII [IU/ml]	0.40-1.52	0.96 ± 0.03	0.91 ± 0.04
Factor XIII [IU/ml]	0.65-1.65	1.01 ± 0.09	0.94 ± 0.06
VWF:RCo [IU/ml]	0.45-1.75	0.95 ± 0.08	1.00 ± 0.01
ADAMTS13 activity [IU/ml]	n.s.	0.91 ± 0.17	0.92 ± 0.09
ADAMTS13 antigen [IU/ml]	n.s.	0.99 ± 0.06	1.00 ± 0.04
Antithrombin [IU/ml]	0.80-1.25	0.92 ± 0.08	0.95 ± 0.13
Heparin cofactor II [IU/ml]	0.65-1.35	0.95 ± 0.02	0.96 ± 0.01
Protein C [IU/ml]	≥ 0.7*	0.93 ± 0.02	0.93 ± 0.01
Protein S [IU/ml]	≥ 0.3*	0.66 ± 0.01	0.55 ± 0.03
Plasmin inhibitor [IU/ml]	≥ 0.2*	0.33 ± 0.03	0.33 ± 0.02
α ₁ -antitrypsin [IU/ml]	n.s.	0.99 ± 0.06	0.99 ± 0.11
C1-inhibitor [IU/ml]	0.60-1.24	0.61 ± 0.02	0.59 ± 0.01
FVIIa [mIU/ml]	25-170	86 ± 5	86 ± 5
TAT [µg/l]	1.0-4.1	2.0 ± 0.4	2.0 ± 0.4
F1+2 [nmol/l]	0.07-0.23**	0.20 ± 0.03	0.20 ± 0.03
D-Dimer [ng/ml]	< 500**	138 ± 18	138 ± 18

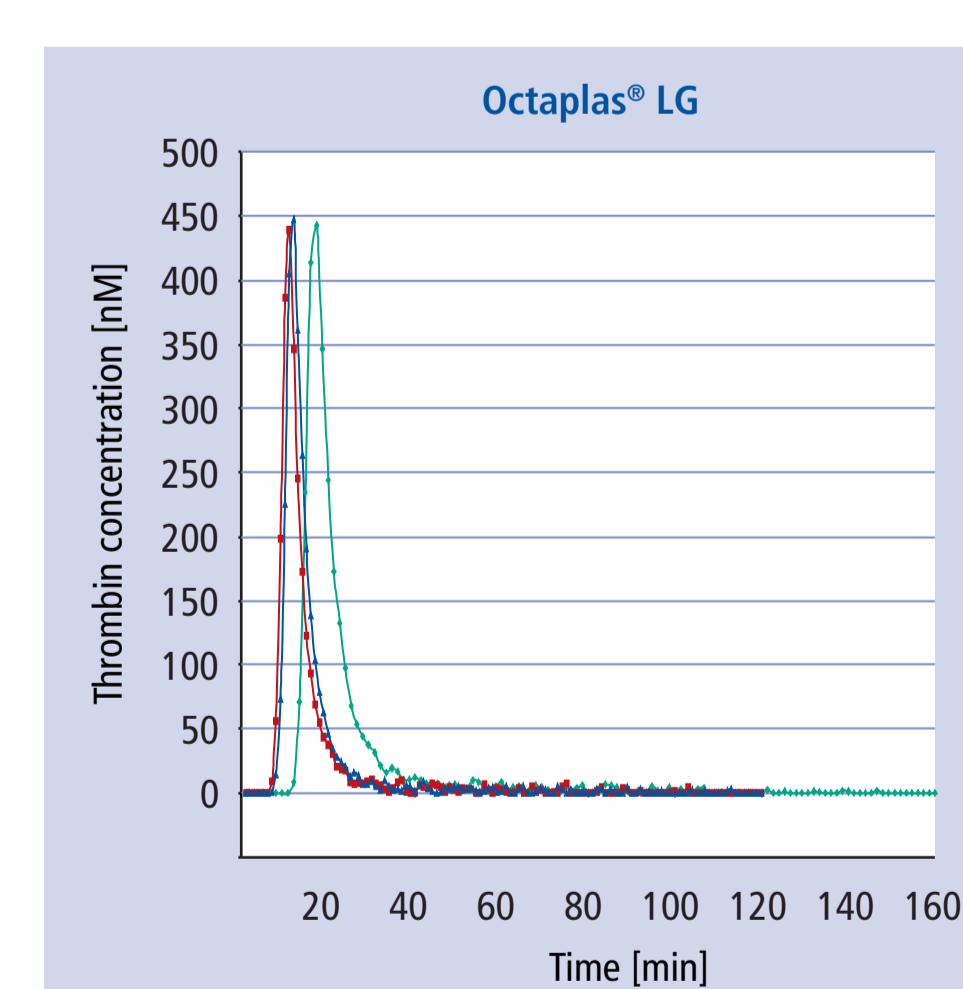
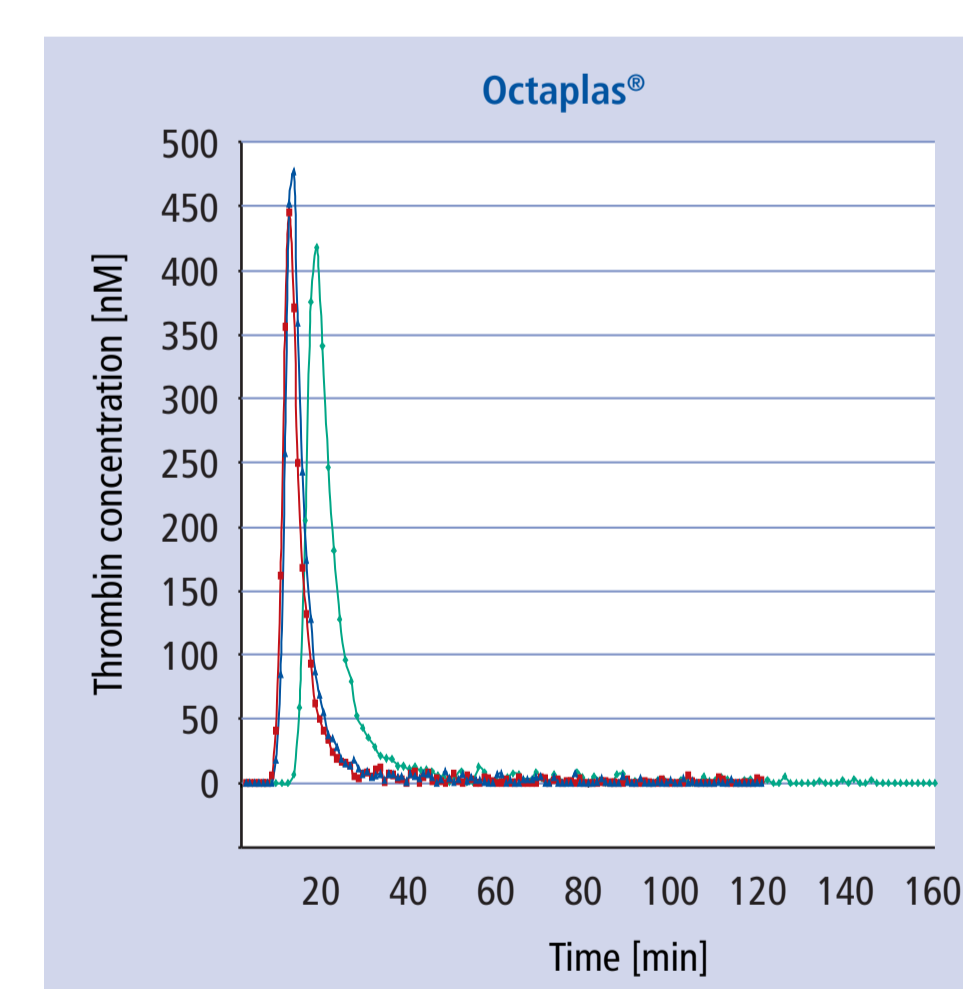
Octaplas® produced with ligand chromatography for specific PrP^{Sc} capture was labelled as Octaplas® LG.

[1] Beek H and Hellstern P, Vox Sang 1998; 74:219-23; n.s., not specified; St. dev., standard deviation

**To be included into the new monograph for S/D plasma (Ph.Eur. 6.1, 07/2008:1646)

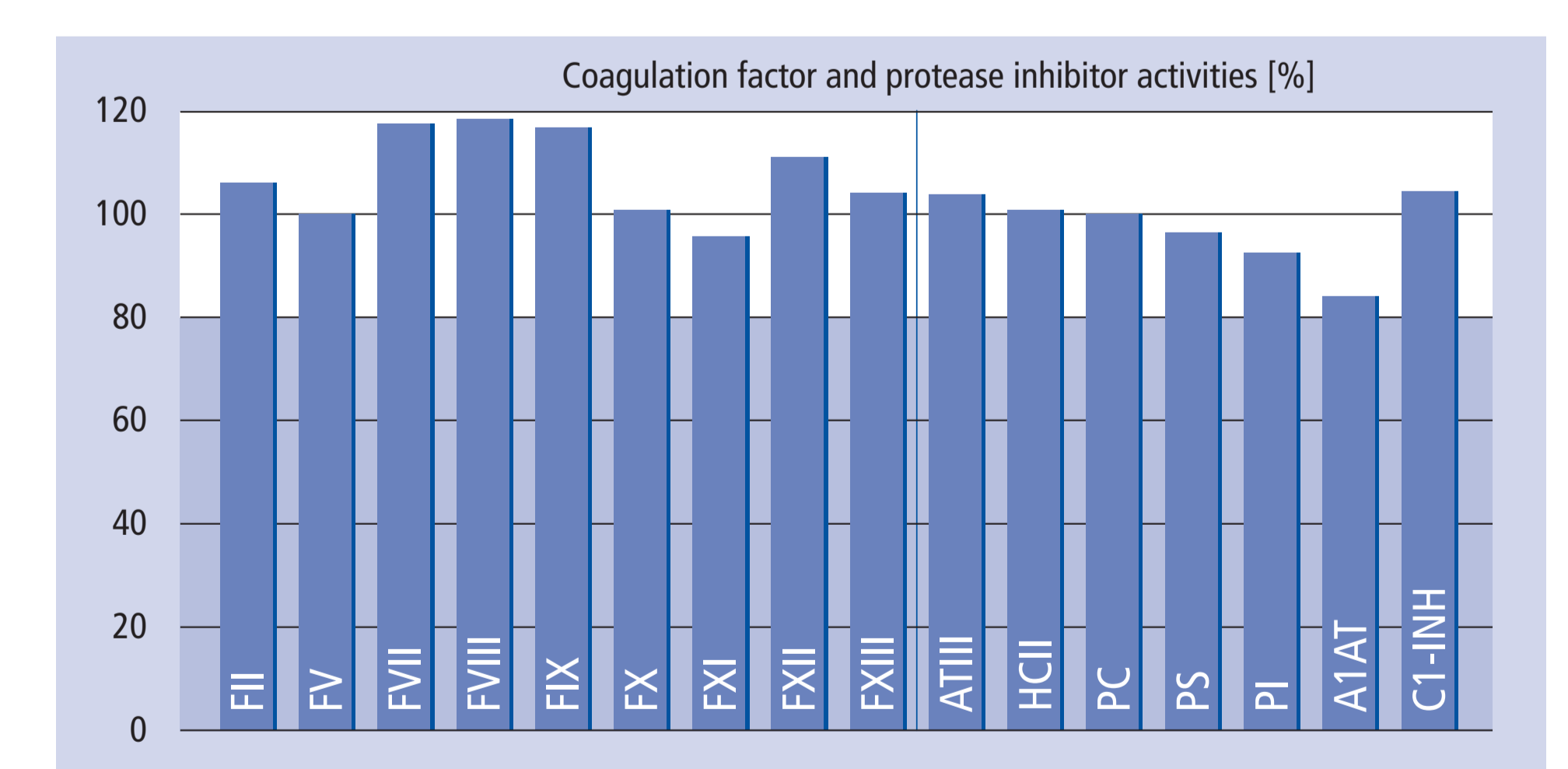
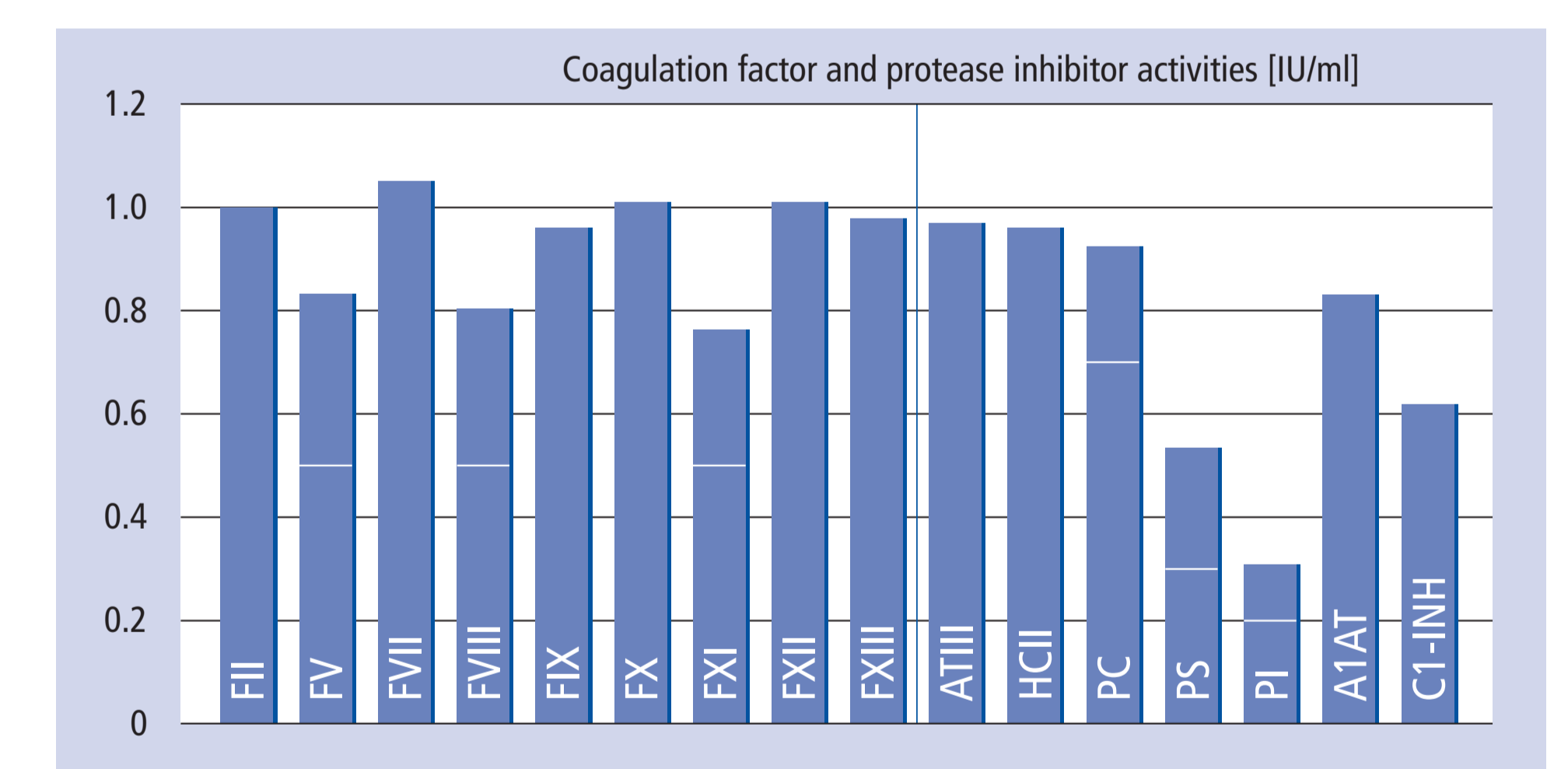
**Reference ranges specified in the ELISA test kits (Enzygnost F1+2, monoclonal and Asserachrom D-Dimer, respectively)

Figure 3: Thrombin generation assay



Results for thrombin concentration and lag time in three consecutive batches of Octaplas® and Octaplas® LG are presented.

Figure 4: Coagulation factor and protease inhibitor activities in Octaplas® LG after one year storage at ≤ -30°C



Mean activities are presented in IU/ml (4/1) and in percent relative to the activities at time point 0 (4/2). Ph.Eur. specification levels for FV, FVIII, FXI, PC, PS and PI [Ph.Eur. 6.2, 07/2008:1646] are indicated (white lines). Biopharmaceutical range (± 20%) is presented (white square).