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Characterization of Thrombate III®, a pasteurized and nanofiltered therapeutic human antithrombin concentrate

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ABSTRACT

Thrombate III® is a highly purified antithrombin concentrate that has been used by clinicians worldwide for more than two decades for the treatment of hereditary antithrombin deficiency. The manufacturing process is based on heparin-affinity chromatography and pasteurization. To modernize the process and to further enhance the pathogen safety profile of the final product, despite the absence of infectious disease transmission, a nanofiltration step was added. The biochemical characterization and pathogen safety evaluation of Thrombate III® manufactured using the modernized process are presented. Bioanalytical data demonstrate that the incorporation of nanofiltration has no impact on the antithrombin content, potency, and purity of the product.

Scaledown models of the manufacturing process were used to assess virus and prion clearance under manufacturing setpoint conditions. Additionally, robustness of virus clearance was evaluated at or slightly outside the manufacturing operating limits. The results demonstrate that pasteurization inactivated both enveloped and non-enveloped viruses. The addition of nanofiltration substantially increased clearance capacities for both enveloped and non-enveloped viruses by approximately 4–6 $\log_{10}$. In addition, the process achieves 6.0 $\log_{10}$ ID_{50} prion infectivity clearance. Thus, the introduction of nanofiltration increased the pathogen safety margin of the manufacturing process without impacting the key biochemical characteristics of the product.

1. Introduction

The Thrombate III® manufacturing process, licensed two decades ago, implemented large-scale double affinity chromatography for the purification of antithrombin (AT; historically referred to as antithrombin III [ATIII]) from pooled human plasma [1,2]. The product is indicated for the treatment of patients with hereditary antithrombin deficiency in connection with surgical or obstetrical procedures or when they suffer from thromboembolism [3]. The original manufacturing process included ethanol precipitation steps, two heparin affinity purification steps, a pasteurization step dedicated to inactivating potentially contaminating viruses, and various connecting steps (Fig. 1) to produce a high-purity antithrombin preparation. Over the two decades of its clinical use, no cases of infectious disease transmission have ever been confirmed for Thrombate III®.

The Thrombate III® manufacturing process flow and design of equipment have recently been modernized to achieve higher efficiency and to incorporate a nanofiltration step to further enhance the pathogen safety profile of the product (Fig. 1). The current report describes the consistent biochemical characteristics of the product before and after the process modernization as well as the pathogen clearance capacity of the modernized process.

2. Materials and methods

2.1. Manufacturing process

The principle steps of the Thrombate III® manufacturing process were previously described [1,2]. Briefly, pooled frozen human

Abbreviations: AT, antithrombin; Thrombate III®, highly purified human antithrombin preparation; BVDV, bovine viral diarrhea virus; CPE, cytopathic effect; HAV, hepatitis A virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus, type 1; ID_{50}, median infectious dose; LRV, $\log_{10}$ virus reduction value; PPV, porcine parvovirus; PRV, pseudorabies virus; Reo3, reovirus type 3; $TCID_{50}$, median tissue culture infectious dose.

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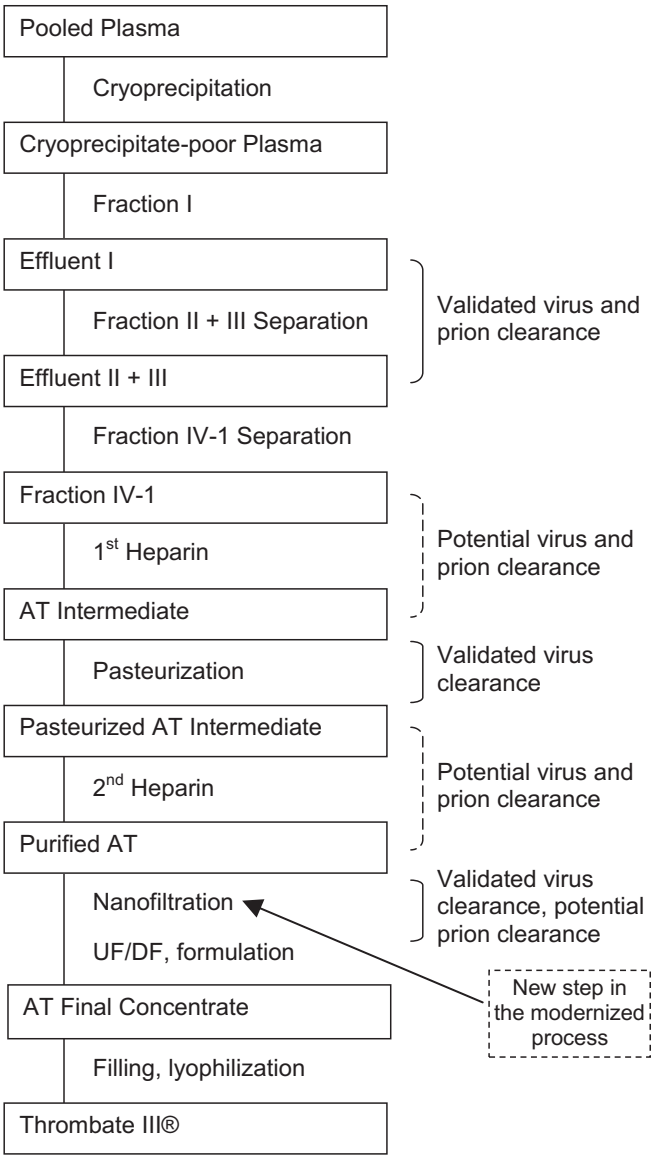

Fig. 1. Flow diagram of the original and modernized Thrombate III® manufacturing processes.

plasma is thawed at low temperature and centrifuged to remove cryoprecipitate. Ethanol is added to the cryoprecipitate-poor plasma to about 8%, and the solution is mixed at low temperature to precipitate Fraction I and generate Effluent I. Next, more ethanol is added to the Effluent I to about 20% to precipitate Fraction II + III and generate Effluent II + III. The Effluent II + III is further processed by adding more ethanol to precipitate Fraction IV-1, which contains antithrombin.

The antithrombin-rich Fraction IV-1 paste is suspended in a slightly basic solution. The antithrombin is purified from the suspension by affinity adsorption to a heparin matrix. The captured antithrombin is washed to remove impurities and then eluted in high salt. The antithrombin intermediate is then heat-treated (pasteurized) in the presence of citrate followed by a second heparin affinity purification step to further remove impurities and the antithrombin denatured during pasteurization. In a new step in the modernized process, the solution of highly purified antithrombin is then filtered using a small pore nanofilter designed to remove potentially contaminating viruses as small as ~20 nm. Subsequently, the material is concentrated using ultrafiltration and diafiltration, formulated, adsorbed by DEAE resin, filtered, and lyophilized to generate the final product (Fig. 1).

2.2. Bioanalytical tests

Antithrombin potency was measured by adding a fixed quantity of thrombin and excess heparin to the sample to form an antithrombin–heparin–thrombin complex. Any remaining thrombin that has not been inhibited by antithrombin would hydrolyze a chromogenic substrate, leading to increased absorbance. Therefore, the absorbance change is inversely proportional to the antithrombin potency in the sample [4].

Antithrombin, alpha-1 acid glycoprotein (AAG), alpha-1 antitrypsin (AAT), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), and albumin levels were determined by immunoprecipitation using infrared nephelometry (Behring Diagnostics, Marburg, Germany) and reagents obtained from Behring.

Antithrombin aggregation was measured using size exclusion high performance liquid chromatography (SE-HPLC). This procedure separates the molecules in solution according to their hydrodynamic volume, related to molecule size, with the largest molecules eluting first and the smallest molecules eluting last. The quantitation is based upon ultraviolet (UV) absorbance at 280 nm, and results are expressed as relative percent area.

2.3. Scaledown models

A scaledown model was developed for each of the manufacturing steps evaluated for pathogen clearance. The bench scale studies for the setpoint evaluation of the cold ethanol fractionation of Effluent I to Effluent II + III process step were carried out at a scale approximately 1/130,000 of manufacturing scale while the robustness studies were carried out at three different scales representing approximately 1/20,000–1/130,000 of the manufacturing scale. A 1/10,000 scaledown model was used to model the pasteurization step of the antithrombin process. The bench scale model used in the evaluation of the nanofiltration step of the antithrombin process utilized a prefilter (1/1650-fold scaledown of the manufacturing process) coupled in line with a nanofilter (1/1200-fold scaledown of the manufacturing process).

The key process parameters of the scaledown models used in the evaluation of pathogen clearance studies were consistent with the manufacturing process. These parameters included temperature, pH, protein concentration, reagent addition rate, intermediate hold-time, filter area, filtration flux, and nanofiltration rinse conditions. Antithrombin potency, total protein, antithrombin specific activity, aggregates and percentage monomer were analyzed to determine if the scaledown models replicated the manufacturing process steps.

2.4. Virus clearance studies

For the virus clearance studies, product intermediates were deliberately spiked with not more than 5% (v/v) of relevant viruses or models for viruses that could potentially contaminate the product. Enveloped viruses studied included human immunodeficiency virus, type 1 (HIV-1), chosen as a relevant blood-borne pathogen, bovine viral diarrhea virus (BVDV), used as a surrogate for hepatitis C virus (HCV), and pseudorabies virus (PRV), which was chosen to model human herpes viruses and other large enveloped DNA viruses. Model non-enveloped viruses studied were reovirus type 3 (Reo3), a non-enveloped virus model, hepatitis A virus (HAV), and porcine parvovirus (PPV), a surrogate for human