

Claims

1. A process for the purification of Prothrombin Complex Concentrate (PCC) from complete plasma or cryo-poor plasma, in particular undiluted cryo-poor plasma, by means of chromatography using a monolithic stationary phase, the method comprising
 - a) performing an initial sample displacement chromatography step of complete plasma or cryo-poor plasma on an anion exchanger to obtain a first fraction enriched in PCC.
2. The process of claim 1, wherein the initial chromatography step is performed with a weak anion exchanger or a strong anion exchanger.
3. The process of claim 1 or 2, wherein the method further comprises
 - b) after elution of the first fraction enriched in PCC from the initial sample displacement chromatography, a second chromatography step of the first fraction enriched in PCC, wherein the chromatography step is performed with an anion exchanger to obtain a second fraction enriched in PCC.
4. The process of claim 3, wherein the method further comprises
 - c) a third chromatography step of the second fraction enriched in PCC, wherein the chromatography step is performed with a cation exchanger to obtain a fraction comprising enriched FIX, and in which fraction other components of the PCC, i.e. FII, FVII, and FX, are depleted or removed.
5. The process of claim 4, wherein the cation exchanger has -SO₃, -SO₄, or -PO₄ groups.
6. The process of any one of the claims 1 to 5, wherein the anion and/or cation exchanger of the second and/or third chromatography step are bulk materials or monolithic materials.
7. The process of any one of the claims 1 or 5, further comprising a solvent/detergent virus inactivation step, preferably wherein the solvent/detergent virus inactivation step is performed prior to the third chromatography step.

8. The process of any one of the claims 1 to 7, wherein a stabiliser is added to at least one of the first, second and third fractions.
9. The process of claim 8, wherein the stabiliser is a positively charged amino acid such as lysine.
10. The process of any one of the claims 1 to 9, wherein a virus filtration step is performed with at least one of the first, second and third fraction.
11. The process of claim 8, wherein heparin is added to at least one of the first, second and third fraction.
12. A fraction comprising FIX obtainable by the process comprising the following steps:
 - (1) providing an undiluted cryo-poor plasma;
 - (2) performing a sample displacement chromatography with the undiluted cryo-poor plasma on a monolithic weak or strong anion exchanger material with about 6 μm pores;
 - (3a) washing the weak or strong anion exchanger monolithic material with a first NaCl buffer having about 100 mM to about 300 mM, in particular about 200 mM NaCl;
 - (3b) followed by washing with a higher second NaCl buffer having about 200 mM to about 350 mM, in particular about 280 mM NaCl;
 - (3c) elution with a NaCl buffer having about 1 M to about 2.5 M NaCl, in particular about 2 M NaCl; thereafter
 - (4) performing a chromatography on a weak or strong anion exchange material; optionally using parts of the fraction obtained after step (4) for PCC manufacturing;
 - (5) stabilization with about 0.1% L-lysine;
 - (6) virus inactivation
 - (7) cation-exchange chromatography;
 - (8) virus filtration on CIM monolith;
 - (9) addition of about 0.1 IU heparin per 1IU FIX,
 - (10) optionally isolating an eluate from the cation exchange material.
13. A fraction comprising PCC obtainable by a process comprising the following steps:
 - (1) providing an undiluted cryo-poor plasma;

- (2) performing a sample displacement chromatography with the undiluted cryo-poor plasma on a monolithic weak or strong anion exchanger material such as DEAE or Q monolithic material with about 6 μm pores;
 - (3a) washing the weak or strong anion exchanger monolithic material with a first NaCl buffer having about 100 mM to about 300 mM, in particular about 200 mM NaCl;
 - (3b) followed by washing with a higher second NaCl buffer having about 200 mM to about 350 mM in particular about 280 mM NaCl;
 - (3c) elution with a NaCl buffer having about 600 mM to about 2.5 M NaCl, in particular about 1.2 M NaCl; thereafter
 - (4) performing a chromatography on a weak or strong anion exchange material such as CIM DEAE chromatography material,
 - (5) optionally isolating an eluate from the weak or strong anion exchange material.
14. The fraction of claim 12 comprising factor IX characterised by a specific activity higher than 120 IU/mg protein, preferably comprising PCC characterised by a clotting factor IX specific activity between 2 and 10 IU/mg protein and correspondingly balanced concentration of both vitamin K dependent clotting factors and inhibitors.