

Appendix I

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PREPARATION OF INTERMEDIATE PURITY FVIII

FOR HEATING: Z8-6-001

PILOT RUN 23.6.86

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PREPARATION OF HEATED INTERMEDIATE PURITY FVIII:PILOT RUN 23.6.861. ZINC PRECIPITATION/A10H3 ADSORPTION

- 1.1 Tris extract (PH 7.0) 5.0 l (sample) *(7.9 u/ml unact sample)*
- 1.2 Titrate slowly from pH 6.99 to pH 6.7
with 0.05 N HCl
- Use vibro mixer
- Volume used 150 mls
- Total Volume 5.150 l (V_1)
- 1.3 Slowly add 333.3 mls Zn Ac solution/l (V_1) 1716.5 mls
- Use vibro mixer
- 1.4 Equilibratin 10 mins at R.T. with gentle mixing
- 1.5 Slowly add 12.5 ~~mg~~ ^{ml} A10H3 suspension/l (V_1) 64.4 mls
- 1.6 Equilibrate 10 mins at R.T. with gentle mixing
- 1.7 Centrifuge 10 mins R.T. 3000 r.p.m. GL
Clarify through u220
Zn/A10H3 supernatant final volume 5.66 l (V_2)
(sample)

2. FORMULATION

- 2.1 Use vibro mixing through out.
- Add a mixture of citrate and calcium chloride to 40mm and 5.5mm respectively.
- 83.4 mls/l (V_2) 0.5m Citrate 472. mls
- 11.1 mls/l (V_2) 0.5m Calcium Chloride 62.8 mls
- 2.2 Add sucrose to 4%
- 40g sucrose/l (V_2) 226.4 g
- 2.3 Add 95mm NaCl
- 5.5g NaCl/l (V_2) 31.1 g

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2.4 Adjust to pH 7.2 using 0.05N NaOH - Omitted

Volume used _____ mls

2.5 Clarify the formulated Zn/A10H3 super through an N B P cartridge 6.194 l (V₃) (sample)

(6.94/ml Urgent sample)

3. ULTRAFILTRATION (UF)

cc ←
Hatched
instructions.

3.1 Concentrate the Zn/A10H3 to 25 iu/ml using the stainless steel Pelicon/LPZA pump system. (sample)

3.2 Final post conc ^{Regenerate} filtration volume 2.134 l (V₄)

3.3 Diafilter the concentrate against 3 volumes (V₃, V₄) of Diafiltration Buffer

Final post DF ^{Regenerate} filtrate volume 2.6 l (sample)
Product back into UF and concentrated to 1.78 l.

4. STERILE FILLING AND DISPENSING

4.1 Clarify the post DF material through an NBP cartridge (sample)

4.2 Sterile filter the clarified material through an NRP cartridge _____ l (sample)

4.3 Dispense 15ml aliquots into 65ml vials with bungs in freeze-drying position.

Total vials number 117

4.4 Distribution ^e the filled vials equally among 4 trays.

4.5 Store 2 trays in the -40 °C cold room and pass the remaining 2 on for freeze-drying.

5. FREEZE DRYING

5.1 Freeze dry the remaining 2 trays of vials in the SM200 controlling the following parameters.

Freezing :- 9hrs -50 °C shelf temp.
Primary Drying

Time	Shelf Temp	Product	Vacuum
24 hrs	-30 °	-28°C	0.10 0.08m barr
12 hrs	-20 °	-20°C	0.10 0.08m barr

Secondary Drying

Time	Shelf Temp	Product	Vacuum
18 24 hrs	+35 °C	+35°C	0.10 0.08m barr and then uncontrolled when product temp = +10°C Vac. reached 0.08 m barr.

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5.2 Stopper cap and seal the vials as normal

5.3 Pass one tray of vials to R & D and the other for heating

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HEATING

6.1 Heat ^{15'} the vials in the Pickstone oven at 80° for 72 hrs

At 24 hrs remove 5' vials

At 48 hrs remove 5' vials

Remove the remainder at 72 hrs.

6.2 Pass the heated vials to R & D on removal from the oven

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4.

PREPARATION OF INTERMEDIATE PURITY FVIII FOR HEATING
PILOT RUN 23.6.86 REAGENTS

4mM ZnAc 20mM Tris pH 6.7

0.05N HCl

0.1 N HCl (made up on the spot from conc HCl)

0.05N NaOH

0.5 M Tri sodium citrate

0.5 M Calcium chloride

Tris (solid)

NaCl (solid)

Sucrose (solid)

Diafiltration (DF) buffer to be made up as follows:- (10l)

20mM Tris - 24.22 g

130mM NaCl - 76g

40mM Tri Sodium Citrate - 834mls of 0.5m stock

5.5mM CaCl₂ - 111mls of 0.5m stock

4% sucrose - 400g

Dissolve in 8l pyrogen free (cooled) water adjust pH to 7.2 using 0.1N HCl.
Adjust final volume to 10l.

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8. PREPARATION OF INTERMEDIATE PURITY FVIII FOR HEATING PILOT RUN 23.6.86 SAMPLES

At each sampling point take:-

3 X 2 ml samples into polypropylene tubes and 1 X 10 ml sample into a polypropylene centrifuge tube.

N.B. at sample point 2.5

i.e. Clarified^a formulated Zn/A10H3 super take an extra 2 ml sample for urgent cogg

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9. OPERATION OF PELICON/LP2A UF SYSTEM
28-6-001 23/686 (2, PT GC 000 05; 10,000 NMWL Cartridges)

1. Connect pump to Pelicon and run Pelicon outlet to waste.
2. Flush out the hypochlorite storage solution with 10l of R.T. PFW.
3. Using 3l of DF Buffer pass 1l through the system to waste and then recirculate with the remaining 2l.
Dead volume 700mls
4. Without draining the DF Buffer from the system connect the Retentate inlet line and Retentate outlet line to the Zn/AlOH₃ super.
5. Begin concentrating the Zn/AlOH₃ super using ^{15'}20 psi air supply to the pump and no back pressure. *(Inlet press. 10psc)*
6. Bring up the back pressure to obtain the required filtrate rate - Do not allow the inlet pressure to exceed 20 psi.
7. Remove the required volumes of filtrate and diafiltrate.
8. On completion of diafiltration flush the system with 10l of 0.5N NaCl using 20 psi air supply and no back pressure.
9. Using 2l of 0.1N NaOH flush 1l to waste and then stand the system in the remaining 1l for 30 mins.
10. Flush out the NaOH with R.T. PFW until pH <8.0.
11. Recirculation 4l of a 1000 ppm dilution of stock hypochlorite for 15 mins.
12. Connect Pelicon outlet and inlet lines to close the system and isolation LP20A pump.
13. Flush the pump with 5l of R.T. PFW; drain and store dry.

28 - 6 - 001 carried out in Sabile.

23-6-86.

1. S.M, fresh TG, made up to UF starting material by N. Doherty - usual joint zinc precipitation and Al anhydrogel.
2. System used: Air pump and stainless steel pellicon - all previously autoclaved set up in Sabile 22-6-86 and left overnight in alahypo. Dead vol = 700ml
3. "Starting vol" = 5660ml post filter - calculation done on this figure.
Actual starting vol = 6194ml to which 700ml wet start should have been added.
4. Aim 3-4 * concn + 3vol diafiltration.
5. A second concentration was required as final vol. was greater than required. This was due to too much diafiltration buffer being added. Recommend that for future expts. the amount of DB be measured out.
6. Details of Run.

Time	vol.	Inlet Pressure psi	Back pressure psi	Air Pump pressure psi	Filtrate Rate ml/min
2.52 pm	wet start	concentration stage.			
2.56	-	12	0	12	164
3.00	1500 ml	15	8	14-15	220
3.13	4000	"	7	14 ⁺	160
3.20 [?]	4760	S.M for diafiltration	1 vol = 1.6 l.		
3.28	1000 ml	15	7 ⁻	14	-
3.38 [?]	2000 ml	15	7 ⁻	14	136
3.45	2 vol (3.2 l)	15	7 ⁻	14	134
3.58	4.8 l = 3 vol	"	"	14 ⁻	126

Final vol = 2.6 l.

Second concentration after 0.5M abcell and 1 l of Diafilt B had been through system.

Final vol to sterile filling = 1780 ml.

SM 200 Z8-6-001

23-26.6.86

Recorder switched on when loading is complete.

Time hrs	Shelf Temp °C	Average Product Temp °C	Vacuum in chamber mbar
0	-47	+2	-
2	-48	-44	-
4	-48	-44	-
6	-48	-44	-
8	-48	-44	-
refrig ends post 9 hrs.			
10	-38	-39	0.14
12	-23	-32	0.125
14	-23	-32	"
16	-23	-32	"
18	"	"	"
20	"	"	"
22	"	"	"
24	"	"	"
26	-24	-32	0.125
28	"	"	"
30	-24	-31	"
32	-24	-30	"
34	-24	-30	"
36	-13	-26	"
38	-13	-26	0.125
40	"	"	"
42	-13	-22	"
44	-13	-19	"
46	-13	-11	"
end of Primary Drying at ~47 hrs.			
48	+24	+6	0.122
50	+36	+32	0.10
52	+38	+36	0.10
54	+39	+38	0.10
56	+39	+39	0.105
58	+40	+40	0.105
60	"	"	"
62	"	"	"
64	"	"	"
nd. 6 1/2	"	"	"

Freezing Time = 9 hrs.

Primary Drying Time = 38 hrs.

Secondary Drying Time = 18 1/2 hrs

