Figure 1: Preparation of VAS203 drug substance

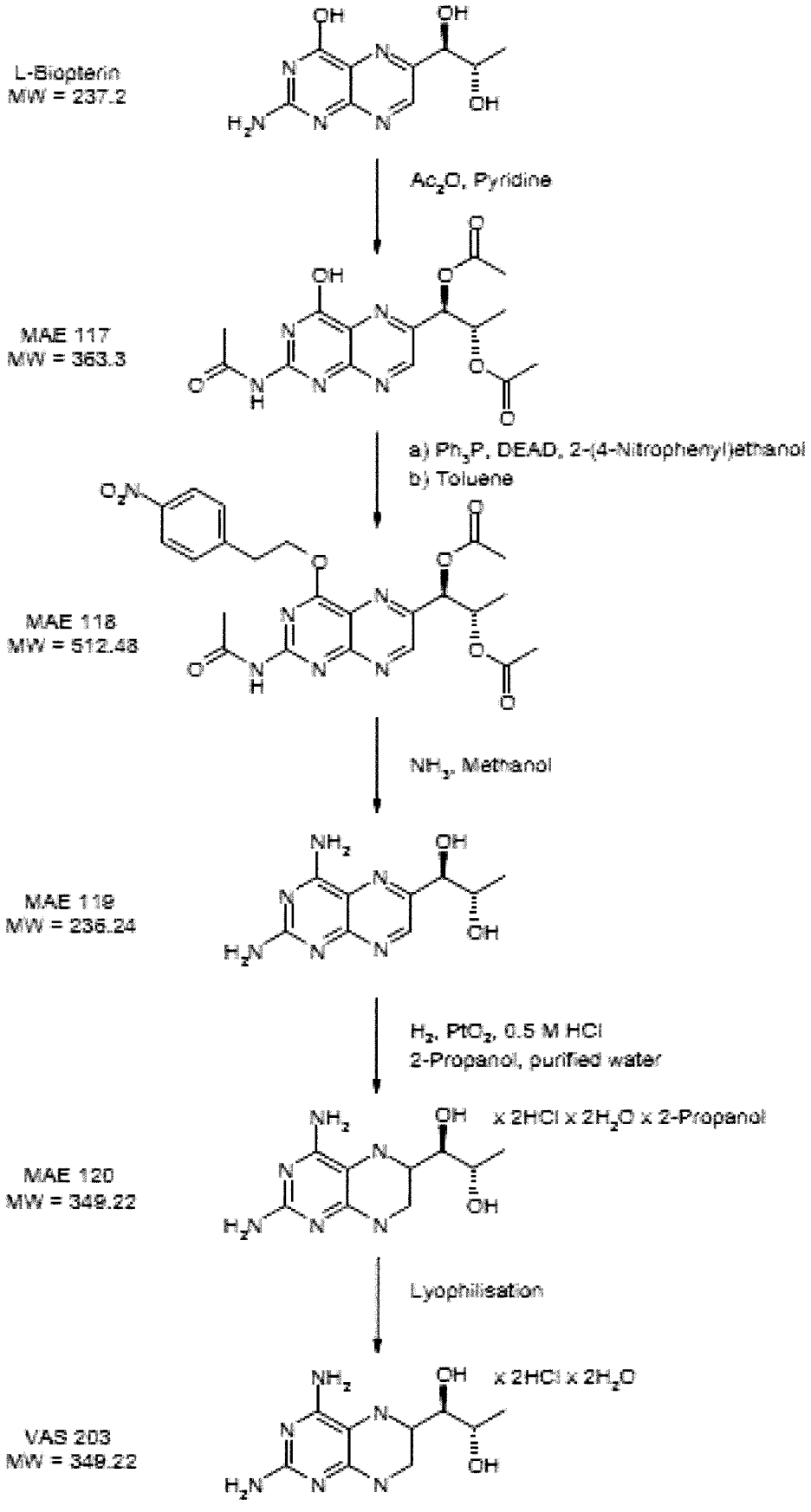


Fig. 2

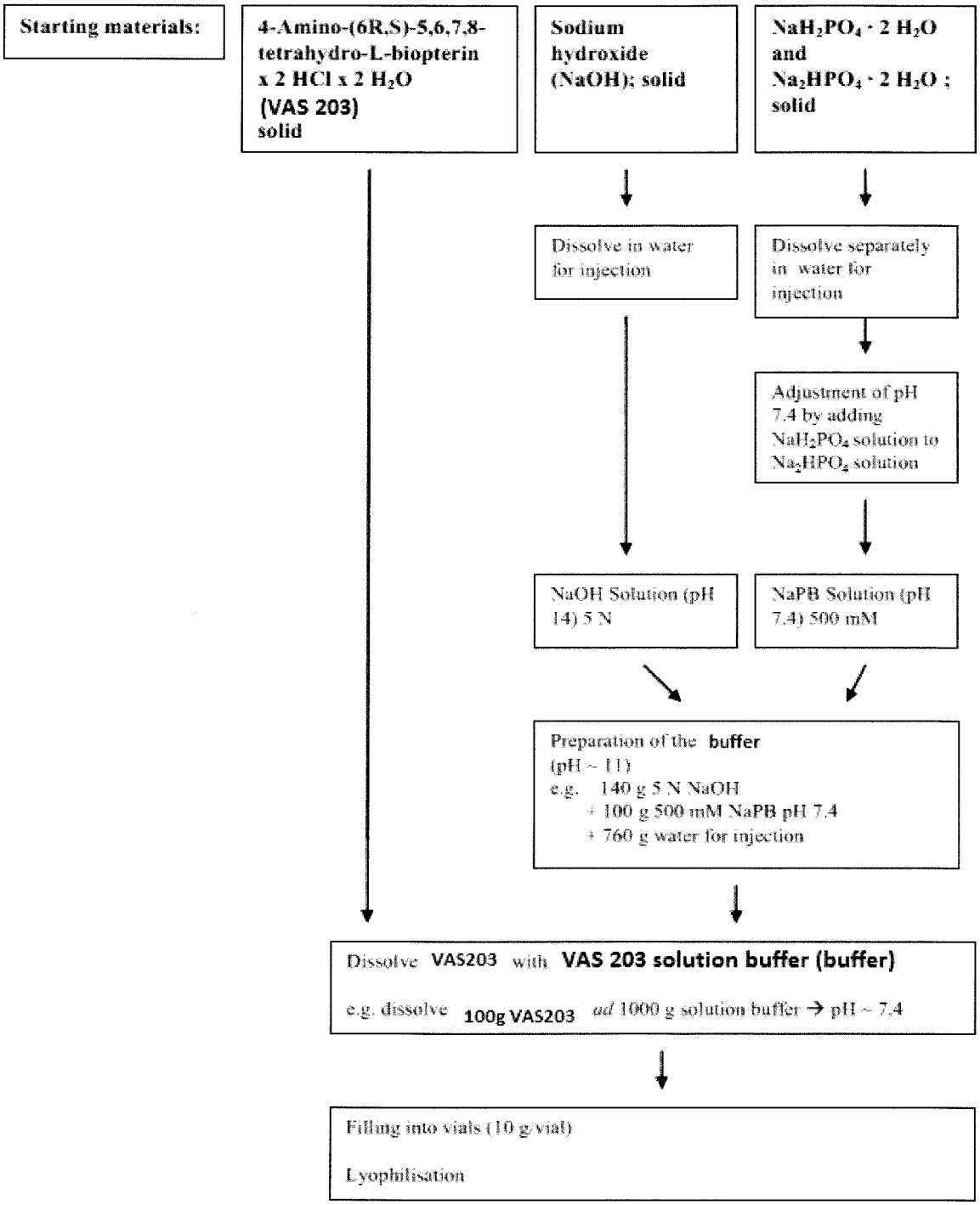

Starting materials:
4-Amino-(6R,S)-5,6,7,8-tetrahydro-L-biopterin x 2 HCl x 2 H_2O (VAS 203) solid
Sodium hydroxide (NaOH); solid
$NaH_2PO_4 \cdot 2\ H_2O$ and $Na_2HPO_4 \cdot 2\ H_2O$; solid
Dissolve in water for injection
Dissolve separately in water for injection
Adjustment of pH 7.4 by adding NaH_2PO_4 solution to Na_2HPO_4 solution
NaOH Solution (pH 14) 5 N
NaPB Solution (pH 7.4) 500 mM
Preparation of the buffer
(pH ~ 11)
e.g. 140 g 5 N NaOH
+ 100 g 500 mM NaPB pH 7.4
+ 760 g water for injection
Dissolve VAS203 with VAS 203 solution buffer (buffer)
e.g. dissolve 100g VAS203 ad 1000 g solution buffer → pH ~ 7.4
Filling into vials (10 g/vial)
Lyophilisation

Fig. 3

Test Item	Specification	Storage Period in month							
		0	3	6	9	12	18	24	36
Appearance	White to pale red or brown lyophilized powder	complies	complies	complies	complies	complies	complies	complies	complies
Identification (HPLC)	Coelution with reference substance	complies	complies	complies	complies	complies	complies	complies	complies
Assay (HPLC) (calculated on anhydrous and chloride-free basis)	650 ± 60 mg	645 mg	649 mg	634 mg	660 mg	684 mg	658 mg	686 mg	694 mg
Purity (HPLC)									
- (6R)-4-Amino-5,6,7,8-tetra-hydro-L-biopterin [% area]	55.5 ± 3.0	54.6	54.6	55.0	54.6	54.4	55.1	54.8	54.6
- (6S)-4-Amino-5,6,7,8-tetra-hydro-L-biopterin [% area]	40.0 ± 3.0	41.5	41.6	41.3	41.6	41.4	41.8	41.5	41.4

Fig. 3 (cont.')

Related substances *(HPLC)*:*												
A [% area]	≤ *4.5*	*2.8*	*2.5*	*2.5*	*2.8*	*3.0*	*2.5*	*2.8*	*2.8*			
B [% area]	≤ *1.0*	*0.2*	*0.2*	*0.3*	*0.2*	*0.2*	*0.2*	*0.2*	*0.2*			
C [% area]	≤ *1.0*	*0.3*	*0.3*	*0.1*	*0.2*	*0.1*	*0.1*	*0.2*	*0.3*			
Further related substances, each [% area]	≤ *0.6*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*			
Further related substances, total [% area]	≤ *2.0*	*0.7*	*0.8*	*0.9*	*0.6*	*0.9*	*0.3*	*0.2*	*0.6*			
Water *(KF-titration)*	*3.0 to 8.0 %*	*5.0 %*	*4.5 %*	*5.1 %*	*4.8*	*4.8 %*	*4.8 %*	*4.8 %*	*4.7 %*			
Sterility	*No evidence of microbial growth*	*complies*	*N.T.*	*N.T.*	*N.T.*	*complies*	*N.T.*	*N.T.*	*complies*			
Endotoxins	≤ *10 I.U./mg*	*complies*	*N.T.*	*N.T.*	*N.T.*	*complies*	*N.T.*	*N.T.*	*complies*			
Particles												
- *Particles ≥ 10 µm*	≤ *6000 / vial*	*600/vial*	*N.T.*	*N.T.*	*N.T.*	*767/vial*	*N.T.*	*N.T.*	*115/vial*			
- *Particles ≥ 25 µm*	≤ *600 / vial*	*3/vial*	*N.T.*	*N.T.*	*N.T.*	*7/vial*	*N.T.*	*N.T.*	*1/vial*			
Reconstituted Solution§												
- *Dissolution Time*	*Max 2 min*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*			
- *Appearance*	*Clear yellowish solution without visible particles*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*	*complies*			
- *pH*	*6.5 to 7.6*	*7.3*	*7.3*	*7.3*	*7.4*	*7.3*	*7.3*	*7.3*	*7.4*			
- *Osmolality [mosmol/kg]*	*260 to 320*	*303*	*295*	*315*	*305*	*320*	*300*	*298*	*305*			

N.T.: Not Tested;

§: Reconstituted in 50 mL WFI

** A: 4-Amino-7,8-dihydro-L-biopterin; B: ∑ (6R)-5,6,7,8-Tetrahydro-L-biopterin and (6S)-5,6,7,8-Tetrahydro-L-biopterin; C: ∑ 1-[(6R)-2,4-Diamino-5,6,7,8-tetrahydropteridin-6-yl]propanol and 1-[(6S)-2,4-Diamino-5,6,7,8-tetrahydropteridin-6-yl]propanol*