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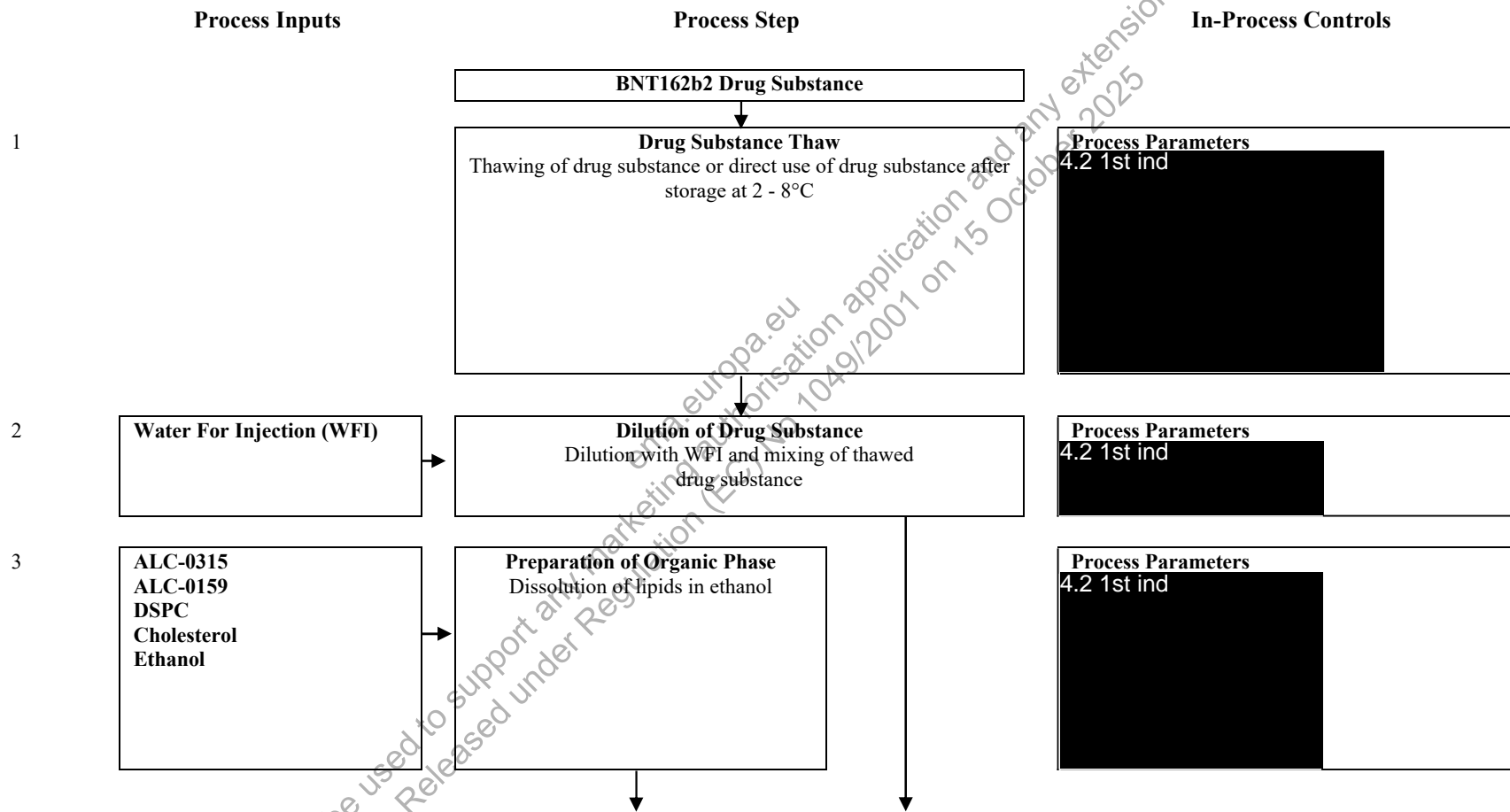
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3.2.P.3.3. LIPID NANOPARTICLE (LNP) PRODUCTION AND BULK DRUG PRODUCT FORMULATION – TRIS/SUCROSE DRUG PRODUCT [BNT MARBURG]

3.2.P.3.3.1. Flow Diagram

The process flow diagram for the BNT162b2 LNP production and Tris/Sucrose bulk drug product formulation manufacturing process is presented in [Figure 3.2.P.3.3-1](#).

Figure 3.2.P.3.3-1. Flow Diagram of the BNT162b2 Tris/Sucrose Drug Product Manufacturing Process and Process Controls



BNT162b2

3.2.P.3.3. Description of Manufacturing Process and Process Controls

LNP Production and Bulk Drug Product Formulation – Tris-Sucrose [BNT Marburg]

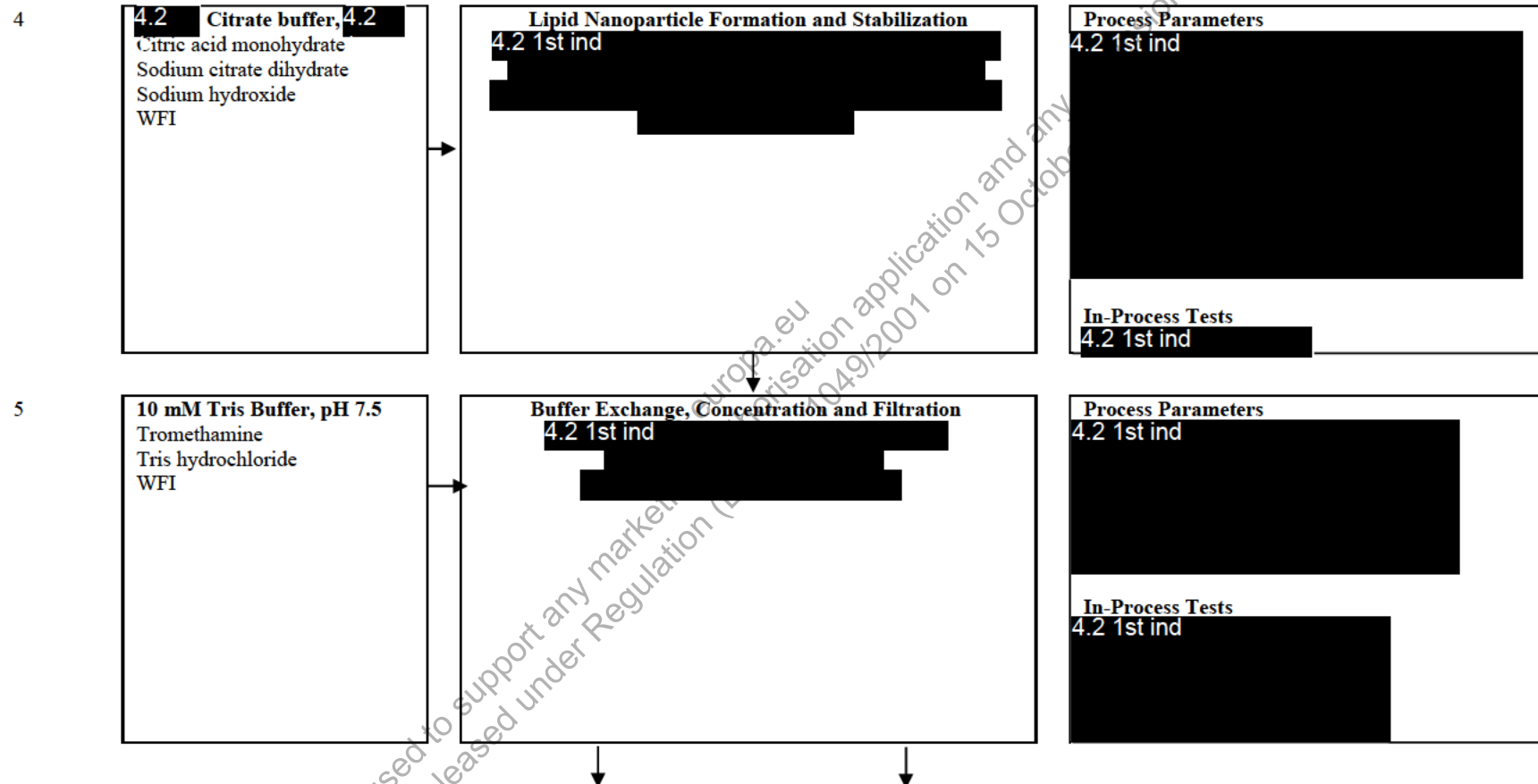
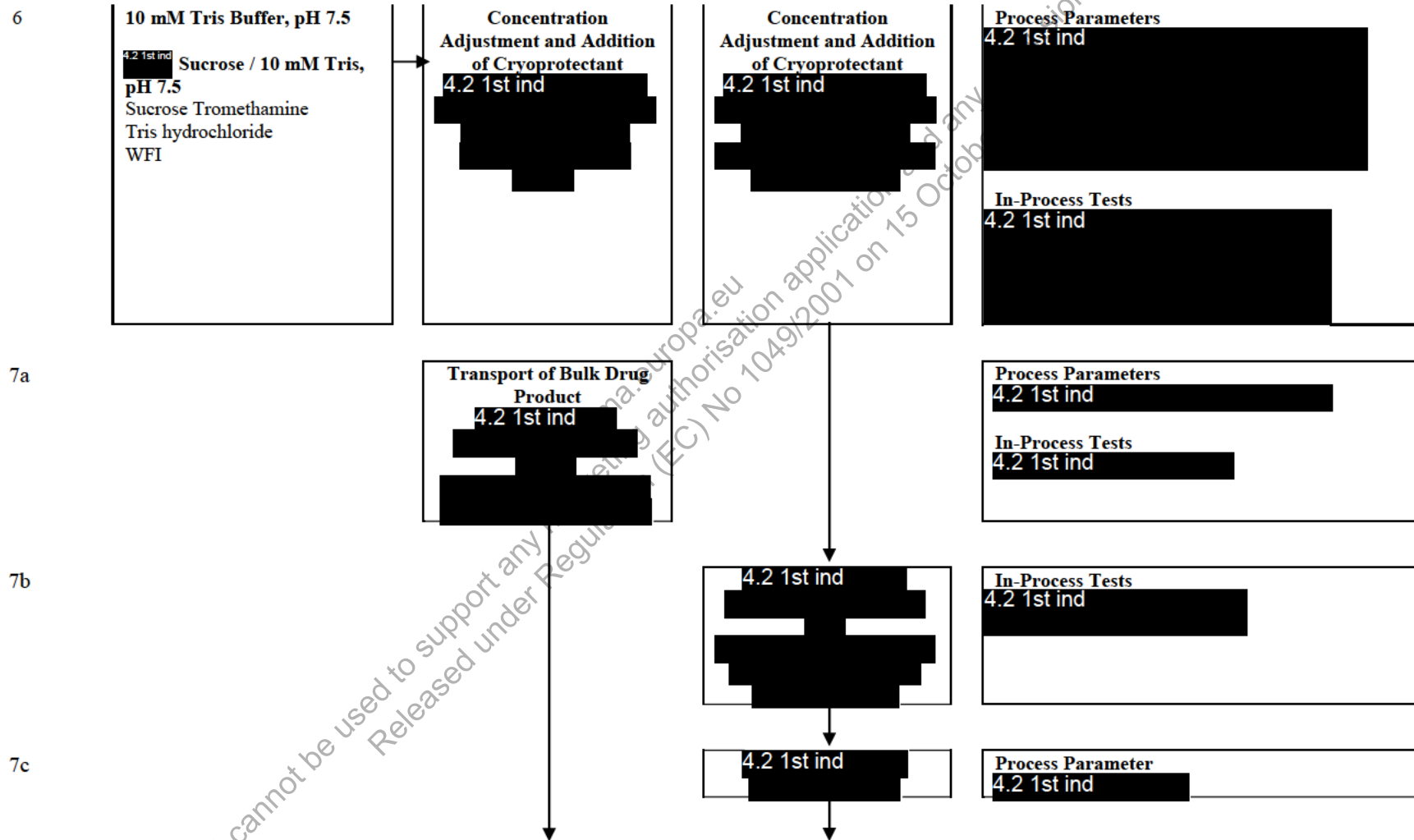
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Figure 3.2.P.3.3-1. Flow Diagram of the BNT162b2 Tris/Sucrose Drug Product Manufacturing Process and Process Controls



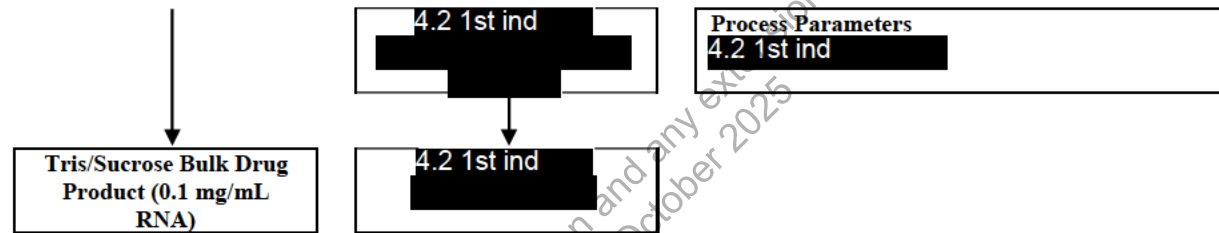
BNT162b2

3.2.P.3.3. Description of Manufacturing Process and Process Controls

LNP Production and Bulk Drug Product Formulation – Tris-Sucrose [BNT Marburg]

Figure 3.2.P.3.3-1. Flow Diagram of the BNT162b2 Tris/Sucrose Drug Product Manufacturing Process and Process Controls

7d



Abbreviations: IPT-C = In-process test for control; IPT-M = In-process test for monitoring; ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; 4.2 1st ind

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3.2.P.3.3.2. LNP Production and Bulk Drug Product Formulation Process Descriptions

Drug substance is stored according to the conditions supported in Section 3.2.S.7.1 Stability Summary and Conclusions.

3.2.P.3.3.2.1. Drug Substance Thaw

Frozen drug substance in ethylene vinyl acetate (EVA) bags may be thawed using controlled thaw equipment which consists of an automated freeze/thaw unit, agitation platform, and a validated thaw recipe. The EVA bags are placed between heat-transfer plates of the freeze/thaw unit. Once thawing is complete, the program ramps the heat transfer fluid (HTF) supply temperature down to a setpoint of 5 °C, at which time the thawed drug substance hold time begins. The controlled thaw equipment remains at a 5 °C setpoint until the EVA bags are removed. The process parameters for controlled thaw of drug substance are summarized in Table 3.2.P.3.3-1.

Table 3.2.P.3.3-1. Process Parameters for Controlled Drug Substance Thaw

Process Parameter	Acceptable Range
4.2 1st ind	

Frozen drug substance EVA bags may also be thawed at controlled room temperature between 15-25 °C. The bags are placed in a controlled temperature room and allowed to thaw. The process parameters for controlled room temperature thaw of drug substance are summarized in Table 3.2.P.3.3-2.

Table 3.2.P.3.3-2. Process Parameters for Controlled Room Temperature Drug Substance Thaw

Process Parameter	Acceptable Range
4.2 1st ind	

Alternatively, the drug substance bags manufactured at the Marburg site are directly used after storage at 2 - 8°C.

3.2.P.3.3.2.2. Dilution of Drug Substance

Thawed drug substance is transferred from EVA bags to a manufacturing vessel. Two to twenty-four drug substance containers from up to seven drug substance batches from a single drug substance supplier may be pooled to achieve the target drug product batch size.

4.2 1st ind

Based on RNA content and weight of drug substance added to the vessel, the Water for Injection (WFI) amount required for dilution to a target concentration of 4.2 1st ind is calculated. The drug substance is diluted with WFI, then mixed until homogenous. The process parameters for dilution of drug substance are summarized in Table 3.2.P.3.3-3.

Table 3.2.P.3.3-3. Process Parameters for Dilution of Drug Substance

Process Parameter	Controlled Setpoint
4.2 1st ind	

3.2.P.3.3.2.3. Preparation of Buffers, Organic Phase and Sucrose Solution**3.2.P.3.3.2.3.1. Preparation of 4.2 1st ind Citrate Buffer, 4.2 1st ind**

The 4.2 1st ind citrate buffer, 4.2 1st ind is compounded or purchased ready to use according to the batch formula shown in Table 3.2.P.3.3-4.

Table 3.2.P.3.3-4. Batch Formula for 4.2 1st ind Citrate Buffer, 4.2 1st ind

Component	Grade	Unit Formula per 1 kg (g)
Citric acid monohydrate	USP, Ph. Eur., JP	4.2 1st ind
Sodium citrate dihydrate	USP, Ph. Eur., JP	
Water for Injection	USP, Ph. Eur.	
Sodium hydroxide	Ph. Eur.	

Abbreviations: q.s. = quantum satis, meaning as much as is sufficient

The citrate buffer is mixed until homogeneous. The pH of the buffer is measured and adjusted to 4.2 1st ind with sodium hydroxide, as necessary. The in-process test for control (IPT-C) for preparation of citrate buffer is summarized in Table 3.2.P.3.3-5. The citrate buffer is filtered with a 4.2 1st ind filter prior to use.

Table 3.2.P.3.3-5. IPT-C Test for 4.2 1st ind Citrate Buffer, 4.2 1st ind

In-process Test	Acceptance Criteria
4.2 1st ind	

- For internally manufactured buffer. Tests and acceptance criteria for external buffer see [Section 3.2.P.3.4 Controls of Critical Steps and Intermediates - In-Process Monitoring and Control – LNP Production and Bulk Drug Product Formulation – Tris/Sucrose \[BNT Marburg\]](#).

3.2.P.3.3.2.3.2. Preparation of 10 mM Tris Buffer, pH 7.5

10 mM Tris buffer, pH 7.5 are compounded or purchased ready to use according to the batch formula shown in Table 3.2.P.3.3-6.

Table 3.2.P.3.3-6. Batch Formula for 10 mM Tris Buffer, pH 7.5

Component	Grade	Unit Formula (g/kg) for 10 mM Tris Buffer
Tromethamine	USP, Ph. Eur.	0.198
Tris Hydrochloride	In-house specification	1.321
Water for Injection	USP, Ph. Eur.	q.s. to 1kg

The 10 mM and 4.2 1st ind Tris buffer, pH 7.5 are mixed until homogeneous and measured for pH. The IPT-C test for preparation of the Tris buffer is summarized in Table 3.2.P.3.3-7.

Table 3.2.P.3.3-7. IPT-C Test for 10 mM Tris Buffer, pH 7.5

In-process Test	Acceptance Criteria
4.2 1st ind	

a. For internally manufactured buffer. Tests and acceptance criteria for external buffer see Section 3.2.P.3.4 Controls of Critical Steps and Intermediates - In-Process Monitoring and Control – LNP Production and Bulk Drug Product Formulation – Tris/Sucrose [BNT Marburg]

The 10 mM Tris buffer, pH 7.5 is filtered with a 4.2 1st ind filter prior to use. The 10 mM Tris buffer, pH 7.5 is used for Concentration Adjustment and Addition of Cryoprotectant (Section 3.2.P.3.3.2.6) of the bulk drug product.

3.2.P.3.3.2.3.3. Preparation of the Lipid Organic Phase

The lipid organic phase is compounded according to the batch formula shown in Table 3.2.P.3.3-8.

Table 3.2.P.3.3-8. Batch Formula for Lipid Organic Phase

Component	Grade	Unit Formula per 1 kg (g)
Ethanol	USP, Ph. Eur.	4.2 1st ind
ALC-0315	In-house specification	
ALC-0159	In-house specification	
DSPC	In-house specification	
Cholesterol	USP, Ph. Eur., JP	

Abbreviations: ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine

The lipids are thawed to 4.2 1st ind, if applicable. Ethanol is added to the organic phase vessel 4.2 1st ind. The lipids are added to the organic phase

vessel 4.2 1st ind

An

excess amount of solution is prepared. 4.2 1st ind

The process parameters for preparation of the organic phase are summarized in Table 3.2.P.3.3-9.

Table 3.2.P.3.3-9. Process Parameters for Preparation of Lipid Organic Phase

Process Parameter	Acceptable Range
4.2 1st ind	

Abbreviations: ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine

3.2.P.3.3.2.3.4. Preparation of 4.2 1st ind Sucrose, 10 mM Tris Solution

The 4.2 1st ind sucrose, 10 mM Tris (Sucrose/Tris) solution is compounded or purchased ready to use according to the batch formula shown in Table 3.2.P.3.3-10.

Table 3.2.P.3.3-10. Batch Formula for 4.2 1st ind Sucrose, 10 mM Tris Solution

Component	Grade	Unit Formula per 1 kg (g)
Sucrose	USP, Ph. Eur., JP	4.2 1st ind
Tromethamine	USP, Ph. Eur.	
Tris Hydrochloride	In-house specification	
Water for Injection	USP, Ph. Eur.	

The Sucrose/Tris solution is mixed until homogeneous and measured for pH. The Sucrose/Tris solution is filtered through a 0.2 µm filter prior to use. The IPT-C test for preparation of the Sucrose/Tris solution is summarized in Table 3.2.P.3.3-11.

Table 3.2.P.3.3-11. IPT--C Tests for 4.2 1st ind Sucrose, 10 mM Tris Solution

In-process Test	Acceptance Criteria
4.2 1st ind	

- For internally manufactured solution. Tests and acceptance criteria for external solution see Section 3.2.P.3.4 Controls of Critical Steps and Intermediates - In-Process Monitoring and Control – LNP Production and Bulk Drug Product Formulation – Tris/Sucrose [BNT Marburg].

3.2.P.3.3.2.4. Lipid Nanoparticle (LNP) Formation and Stabilization

4.2 1st ind

The process parameters for formation and stabilization of lipid nanoparticles is summarized in Table 3.2.P.3.3-12.

Table 3.2.P.3.3-12. Process Parameters for Formation and Stabilization of LNPs

Process Parameter	Acceptable Range
4.2 1st ind	

Abbreviation: LNP = lipid nanoparticle

3.2.P.3.3.2.5. Buffer Exchange, Concentration and Filtration

3.2.P.3.3.2.5.1. Buffer Exchange and Concentration

For the 4.2 1st ind batch size, one tangential flow filtration (TFF) filter with a surface area of 4.2 1st ind and for the 4.2 1st ind batch size, two TFF filters with a total surface area 4.2 1st ind are used.

To prepare for the Buffer Exchange and Concentration operation, the TFF membranes are flushed 4.2 1st ind for equilibration. When membranes are re-used between lots, the membrane and fluid path are sanitized 4.2 1st ind prior to equilibration 4.2 1st ind.

4.2 1st ind

Membranes that will be reused are cleaned 4.2 1st ind

The filter information for buffer exchange and concentration is summarized in Table 3.2.P.3.3-13.

Table 3.2.P.3.3-13. Filter Properties for Buffer Exchange and Concentration

4.2 1st ind

The process parameters for tangential flow filtration are summarized in Table 3.2.P.3.3-14.

Table 3.2.P.3.3-14. Process Parameters for Buffer Exchange and Concentration

Process Parameter	Acceptable Range
4.2 1st ind	

3.2.P.3.3.2.5.2. Filtration

Samples are taken for bioburden in-process testing prior to bioburden reduction filtration.

The formulated drug product suspension is filtered through a bioburden reduction filter into a transport vessel 4.2 1st ind

3.2.P.3.3.2.6. Concentration Adjustment and Addition of Cryoprotectant

Concentration adjustment can be carried out 4.2 1st ind :

- 4.2 1st ind
- 4.2 1st ind

3.2.P.3.3.2.6.1. Bulk Drug Product (0.1 mg/mL)

Samples are taken for 4.2 1st ind prior to formulation of the bulk drug product. This in-process measurement of 4.2 1st ind is used to calculate the final batch weight and thereby the amount of 4.2 sucrose, 10 mM Tris solution and Tris buffer required to achieve the target drug product RNA content of 0.1 mg/mL and 300 mM sucrose.

4.2 1st ind

The calculated weights of 1) Tris buffer, pH 7.5 and 2) of 10 mM Tris, 4.2 sucrose solution are added to ensure a target concentrations of 0.1 mg/mL RNA and 300 mM sucrose. The solution is mixed until homogeneous.

The process parameters for concentration adjustment and addition of cryoprotectant are summarized in Table 3.2.P.3.3-15.

Table 3.2.P.3.3-15. Process Parameters for Concentration Adjustment and Addition of Cryoprotectant

Process Parameter	Acceptable Range
4.2 1st ind	

3.2.P.3.3.2.6.2. Drug Product Intermediate (0.5 mg/mL)

4.2 1st ind results in a DPI solution with an RNA concentration of 0.5 mg/mL .

Samples are taken for 4.2 1st ind prior to addition of buffer solutions. This in-process measurement of 4.2 1st ind is used to calculate the amount of 4.2 sucrose, 10 mM Tris solution and Tris buffer required to achieve the target RNA content of 0.5 mg/mL and 300 mM sucrose.

4.2 1st ind

The calculated weights of 1) Tris buffer, pH 7.5 and 2) of 10 mM Tris, 4.2 sucrose solution are added to ensure a target concentrations of 0.5 mg/mL RNA and 300 mM sucrose. The solution is mixed until homogeneous.

The process parameters for concentration adjustment and addition of cryoprotectant are summarized in Table 3.2.P.3.3-16.

Table 3.2.P.3.3-16. Process Parameters for Concentration Adjustment to 0.5 mg/mL and Addition of Cryoprotectant

Process Parameter	Acceptable Range
4.2 1st ind	

3.2.P.3.3.2.7. Transport

3.2.P.3.3.2.7.1. Bulk Drug Product (0.1 mg/mL)

Prior to transport, the bulk drug product is filtered through 4.2 1st ind filter and filled into flexible containers (FCs) having a volume of 4.2 1st ind

The FCs are stored at 2 - 8 °C until transport (shipping) to the Fill and Finish site and shipped at this temperature to the fill and finish site. The process parameters for transport of bulk drug product are summarized in Table 3.2.P.3.3-17.

Table 3.2.P.3.3-17. Process Parameters for Transport of Bulk Drug Product

Process Parameter	Acceptable Range
Storage and shipment temperature liquid bulk ^a	2 - 8 °C

a. For bulk drug product to be immediately processed at the fill and finish site.

3.2.P.3.3.2.7.2. Drug Product Intermediate (0.5 mg/mL)

The DPI with RNA concentration of 0.5 mg/mL is filtered 4.2 1st ind and filled into FFT bags having a volume of 4.2 1st ind. A sample for determination of the 4.2 1st ind is taken before dispensing.

The DPI is stored and shipped at -80 to -60 °C at BioNTech Marburg (Section 3.2.P.3.4 Controls of Critical Steps and Intermediates – Drug Product Intermediate 0.5 mg/mL – Stability Summary and Conclusion – Tris/Sucrose). The process parameters for the DPI are outlined in Table 3.2.P.3.3-18.

Table 3.2.P.3.3-18. Process Parameters for Storage and Transport of DPI (0.5 mg/mL) at BioNTech Marburg

Process Parameter	Acceptable Range
Storage and shipment temperature	-80 to -60 °C

3.2.P.3.3.2.8. Hold Times

The hold times of the in-process materials during LNP formation and bulk drug product formulation are provided in Table 3.2.P.3.3-19.

Table 3.2.P.3.3-19. LNP Production and Bulk Drug Product Formulation Process Hold Times

Material or In-Process Hold Description ^a	Process Steps	Target Hold Time
Drug substance thaw	Controlled thaw equipment: Time drug substance in ethylene vinyl acetate (EVA) containers is thawed 4.2 1st ind	4.2 1st ind
	Controlled room temperature thaw: Time drug substance in EVA containers is thawed at controlled room temperature.	
Drug substance post thaw	Maximum time that thawed BNT162b2 drug substance can be held at 15-25 °C and 2-8 °C in the ethylene vinyl acetate (EVA) container including addition of DS to the vessel up to the point of dilution.	
Drug substance post dilution at 4.2	Time from addition of 4.2 to drug substance until end of LNP formation step	
Organic phase post mix at 4.2 1st	Time from end of organic phase mixing until end of LNP formation.	
LNP formation	Time from start of mixing aqueous and organic phases 4.2 1st ind until start of 4.2 step including collection and hold at 4.2 1st	
Time of 4.2 1st in filtration unit operation 4.2	Time from start of 4.2 operation to end of 4.2 1st ind filtration while product is at 4.2 1st	
Time post first 4.2 filtration at 4.2 1st	Time post 4.2 1st ind filtration until end of 4.2 1st ind filtration with 4.2 1st until the start of concentration adjustment and cryoprotectant addition.	

a. Temperature values of 15-25 °C represent room temperature

4.2 1st ind

4.2 1st ind

Abbreviations: RT = Room temperature; WFI = Water for Injection; LNP = lipid nanoparticle; TFF = Tangential flow filtration.