

TABLE OF CONTENTS

LIST OF TABLES	1
LIST OF FIGURES	1
3.2.P.2.3. DEVELOPMENT HISTORY	2
3.2.P.2.3.1. Demonstration of Comparability	5
3.2.P.2.3.1.1. Process Design Comparability	5
3.2.P.2.3.1.2. Comparability of Product Quality	10
3.2.P.2.3.1.2.1. Discussion of Release and Characterization Data to Support BNT162b2 Product Quality Comparability	19
3.2.P.2.3.1.2.2. Additional Heightened Characterization Profiles to Support BNT162b2 Product Quality Comparability	20
3.2.P.2.3.1.3. Overall Conclusions for Comparability	24

LIST OF TABLES

Table 3.2.P.2.3-1. BNT162b2 Drug Product Development History	4
Table 3.2.P.2.3-2. Comparison of Drug Product Manufacturing Processes (Multiple Markets)	6
Table 3.2.P.2.3-3. BNT162b2 Comparability of Release Test Results	11
Table 3.2.P.2.3-4. BNT162b2 Clinical and Emergency Supply Drug Product Lots	15
Table 3.2.P.2.3-5. BNT162b2 Drug Product Comparability Testing Panel	16
Table 3.2.P.2.3-6. Comparability Data for BNT162b2 Drug Product Lots	17

LIST OF FIGURES

Figure 3.2.P.2.3-1. ¹ H NMR Spectra for BNT162b2 Drug Product Lots	21
Figure 3.2.P.2.3-2. 5'-Cap RP-HPLC Chromatograms for BNT162b2 Drug Product Lots	23
Figure 3.2.P.2.3-3. Poly(A) Tail RP-HPLC Chromatograms for BNT162b2 Drug Product Lots	24

3.2.P.2.3. DEVELOPMENT HISTORY

The BNT162b2 drug product is a preservative-free, sterile, multi-dose concentrate of RNA-containing lipid nanoparticles (LNPs) formulated in phosphate-buffered saline (PBS) and 300 mM sucrose at pH 7.4 to be diluted for intramuscular administration. The drug product has been developed to meet the quality target product profile as described in [Section 3.2.P.2 Introduction](#). The LNP and drug product formulations and processes have remained the same throughout development, except for necessary changes to the scale as development has progressed from initial clinical supplies to commercial manufacture. Based on evaluation of the results of initial clinical trials, which included multiple RNA constructs, the RNA BNT162b2 construct has been chosen for commercial supply. Development history for the RNA drug substance is discussed in [Section 3.2.S.2.6 Development History and Comparability Assessment](#).

The initial LNP and drug product formulation processes were developed at Acuitas Therapeutics, and scale-up and manufacture were performed at Polymun Scientific for clinical trial material and emergency supply (emergency use in the United States). The process has been transferred to Pfizer commercial facilities in Kalamazoo, MI, USA, and Puurs, Belgium, for manufacture of later clinical materials (Puurs), emergency supply and commercial supply. Commercial supply production facilities may vary on a market-by-market basis (see [Section 3.2.P.3.1 Manufacturer\(s\)](#) for registered facilities applicable to this dossier), however the processes are highly aligned and therefore a global view is presented within the development sections of the registration dossier. Changes to accommodate scale-up and facility fitting have been made; for example, the aqueous and organic phase concentrations and flowrates for LNP fabrication were changed from a “classical” process to an “upscale” process to enable higher process throughput, combined with “scale out” flexibility of parallel processing using multiple T-mixers. The scale of the tangential flow filtration (TFF) equipment was similarly adapted for larger batch volumes and higher process throughput. For manufacture of clinical and emergency supply, RNA drug substance was subjected to freeze in [4.2 1st ind](#) bags in temperature-controlled freezers for storage at -20 °C at BioNTech IMFS with subsequent thaw at controlled room temperature at Polymun. For additional emergency supply and commercial manufacture, the freezing process to -20 °C for drug substance in [4.2 1st ind](#) bags may utilize programmed controlled freeze equipment or temperature-controlled freezers at the commercial drug substance manufacturing site, followed by thaw using programmed controlled thaw equipment or controlled room temperature thaw at drug product manufacturing sites. The development of the BNT162b2 LNP and drug product manufacturing process heavily leveraged prior knowledge from the RNA-delivery platform development at Acuitas Therapeutics and fill/finish of other sterile (aseptically manufactured) and biologic drug products at Pfizer.

The changes to the manufacturing process have been driven by operational efficiency, and, during facility transfer, driven by fit-to-facility equipment, leading to the proposed commercial process. An overall summary of major process changes made for the LNP fabrication and formulation and filling processes during development is provided in

[Table 3.2.P.2.3-1](#). The indicated process changes are not expected to impact the overall product quality of the resulting drug product lots.

090177e195a3950d\Approved\Approved On: 28-Nov-2020 06:08 (GMT)

Table 3.2.P.2.3-1. BNT162b2 Drug Product Development History

Use		Phase 1/2/3 CTM	Phase 2/3 CTM	Emergency Supply ^a	Commercial Supply ^c	Rationale
Input DS process		Process 1	Process 1 and/or Process 2	Process 2		Drug substance process adapted to increased scale.
DP concentration		4.2 1st ind				No change
DP formulation matrix		300 mM Sucrose, PBS pH 7.4				No change
DS/LNP batch size		4.2 1st ind “classical”	4.2 1st ind “classical”	4.2 1st ind “upscale”	4.2 1st ind “upscale”	Production scale up for increased supply
DP lot size (vials)		~1300-50,000	Up to 80,000	Up to 200,000	Up to 309,000	
DP fill volume		4.2 1st ind		0.45 mL		DP optimized in response to clinical data. Formulation unchanged.
Preparation for administration		Syringe to syringe or in-vial dilution	In-vial dilution			DP optimized for dosing.
Sources for lipids	ALC-0315	Avanti		Avanti or Croda	Croda	Additional vendors for increased supply.
	ALC-0159	Avanti		Avanti	Avanti	
	DSPC	Lipoid		Lipoid or Avanti	Avanti	
	Cholesterol	Evonik		Evonik or Avanti	Evonik or Avanti	
LNP fabrication site		Polymun, Austria			Kalamazoo, MI, USA Puurs, Belgium	Increased capability for routine manufacture.
DP fill site		Polymun, Austria	Polymun, Austria Puurs, Belgium (S2F2) ^c	Puurs, Belgium ^d	Kalamazoo, MI; USA Puurs, Belgium ^d	Increased capability for routine manufacture.
DP/LNP release test sites		Polymun, Austria		Andover, MA, USA Chesterfield, MO, USA	Kalamazoo, MI, USA Puurs, Belgium Andover, MA, USA Chesterfield, MO, USA	Addition of sites for routine testing.

a. For US use only. Material may also be used for Phase 2/3 CTM

b. Fill volume adjusted per clinical dose decisions

c. Clinical manufacturing line

d. Two commercial manufacturing lines used: WSL5 for emergency supply and FC2 for commercial supply

e. Not all manufacturing sites will be registered in all markets. See [Section 3.2.P.3.1 Manufacturer\(s\)](#) for registered facilities applicable to this dossier.

Abbreviations: CTM = clinical trial material; DS = drug substance; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; DP = drug product; LNP = lipid nanoparticle

Lipid Vendors: Avanti = Avanti Polar Lipids, Alabaster, AL USA; Croda = Croda Europe, Ltd, Staffordshire, UK; Lipoid = Lipoid GmbH, Ludwigshafen, Germany; Evonik = Evonik Corporation (formerly Wilshire Technologies, Inc.), Princeton, NJ USA and Birmingham, AL, USA

3.2.P.2.3.1. Demonstration of Comparability

A comprehensive plan for demonstration of comparability among clinical supplies and commercial product including an assessment of the process designs and comprehensive characterization of the resulting product quality is planned. Data for this section is pending and will be updated once the data has been generated, analyzed, and verified. See table “Schedule for Additional CMC Content Availability” in [Mod 2.3 Quality Overall Summary Introduction](#).

3.2.P.2.3.1.1. Process Design Comparability

Clinical supplies were initially produced entirely at Polymun, Austria. The first transition in DP manufacturing location involved retaining LNP fabrication at Polymun, followed by transport of the fully formulated LNPs (bulk drug product prior to sterile filtration) to Pfizer Puurs, Belgium for fill/finish operations. Fill/finish operations at Puurs were initially conducted on the S2F2 line with transition to the WSL5 line for larger batch volumes. For routine global commercial supply production, the LNP fabrication process has been fully transferred to multiple commercial production facilities, including both Pfizer, Kalamazoo, MI (USA) and Pfizer, Puurs, Belgium with fill/finish at the same sites. As a global view, two lines are being validated at Kalamazoo (Line 8 and Line 18) and one line is currently being validated at Puurs (FC2), though additional lines are planned for future validation and may be registered in the future in some markets. For routine commercial production, not all sites will be registered in all markets; see [Section 3.2.P.3.1 Manufacturer\(s\)](#) for relevant facilities applicable to this dossier.

An assessment of minor process adjustments made for fit-to-facility and equipment is summarized in [Table 3.2.P.2.3-2](#).

Table 3.2.P.2.3-2. Comparison of Drug Product Manufacturing Processes (Multiple Markets)

Process Step	Clinical Supplies	Emergency Supply	Commercial Supply (Multiple Markets)			Assessment
LNP Fabrication Site	Polymun	Polymun	Kalamazoo	Puurs	Puurs	Overall site capabilities are similar. Overall process steps and flow are the same for all sites. Commercial site processes to be fully validated.
DP Fill Finish Site	Polymun, Puurs	Puurs	Kalamazoo	Puurs	Puurs	Comparability among RNA drug substances is addressed in Section 3.2.S.2.6 Development History and Comparability Assessment
DS Site	BioNTech, IMFS	Andover, BioNTech Mainz / Rentschler	Andover	Andover	BioNTech Mainz / Rentschler	Product contact layer is identical for both ^{4.2 1st ind} containers (Section 3.2.S.6 Container Closure). No impact to quality expected from thaw process (clinical supplies used uncontrolled thaw, which is considered worst case).
Drug Substance Thaw	DS container: ^{4.2 1st ind} controlled room temperature thaw	DS container: ^{4.2 1st ind} controlled room temperature thaw	DS container: ^{4.2 1st ind} controlled thaw or controlled room temperature thaw	DS container: ^{4.2 1st ind} controlled thaw or controlled room temperature thaw	DS container: ^{4.2 1st ind} controlled room temperature thaw	Concentration increased to enable process scale up and efficiency. Addition of citrate buffer moved to in-line dilution in LNP Formation and Stabilization step for improved stability of diluted DS during hold time.
Dilution of DS	^{4.2 1st ind}					

Table 3.2.P.2.3-2. Comparison of Drug Product Manufacturing Processes (Multiple Markets)

Process Step	Clinical Supplies	Emergency Supply	Commercial Supply (Multiple Markets)			Assessment
Preparation of Organic Phase	ALC-0315 and ALC-0159 from Avanti, DSPC from Lipoid, cholesterol from Evonik (Wilshire). 4.2 1st ind	ALC-0315 from Avanti or Croda, ALC-0159 from Avanti, DSPC from Lipoid or Avanti, cholesterol from Evonik (Wilshire) or Avanti. 4.2 1st ind	All lipids from Avanti (and Croda ^a for ALC-0315, Evonik for cholesterol) 4.2 1st ind	All lipids from Avanti (and Croda ^a for ALC-0315, Evonik for cholesterol) 4.2 1st ind	All lipids from Avanti (and Croda ^a for ALC-0315, Evonik for cholesterol) 4.2 1st ind	Comparability of ALC-0315, DSPC and cholesterol from different vendors will be established at the raw material level and with lab scale LNP assessment. No change in ALC-0159 supply. Concentration increased to enable process scale up and efficiency.
LNP Formation & Stabilization	One T-mixer used. Classical process flow rate 4.2 1st ind	One to four T-mixers. Upscale process flow rate increased flow rates 4.2 1st ind	Eight T-mixers. Increased flow rates 4.2 1st ind Citrate buffer added in-line during LNP Formation and Stabilization step.	Eight T-mixers. Increased flow rates 4.2 1st ind Citrate buffer added in-line during LNP Formation and Stabilization step.	Eight T-mixers. Increased flow rates 4.2 1st ind Citrate buffer added in-line during LNP Formation and Stabilization step.	Ratio of aqueous phase to lipid phase is maintained. Increased flow rates for process efficiency. All critical quality attributes are maintained.
Concentration, Buffer Exchange, Filtration	TFF membrane area 4.2 1st ind	TFF membrane area 4.2 1st ind	TFF membrane area 4.2 1st ind	TFF membrane area 4.2 1st ind	TFF membrane area 4.2 1st ind	Increased TFF loading for increased batch size/capacity. Same diavolumes and concentrations maintained. No impact to quality attributes.
Concentration Adjustment & Addition of Cryoprotectant	Varied batch volume.	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	Process scale increased for commercial production efficiency.

Table 3.2.P.2.3-2. Comparison of Drug Product Manufacturing Processes (Multiple Markets)

Process Step	Clinical Supplies	Emergency Supply	Commercial Supply (Multiple Markets)			Assessment
Bioburden reduction filtration	Optional in case of transport. For fill finish at Puurs, included with filling of flexible containers (FCs) prior to shipment.	Included with filling of flexible containers (FCs) prior to shipment.	Not required. (filtration included as input to prior step, no transport)	Not Required (filtration included as input to prior step, no transport)	Not Required (filtration included as input to prior step, no transport)	Filtration step not required for commercial process as there is no transport of bulk drug product from site to site. Microbial control maintained as all process streams are 0.2 µm filtered in prior step.
Hold or Shipping condition	Polymun fill/finish: Hold in FCs 4.2 1st ind Puurs fill/finish: Hold and Shipment in FCs 4.2 1st ind at 2-8 °C 4.2 1st ind	Hold and Shipment in FCs 4.2 1st ind at 2-8 °C	Hold in Stainless Steel, chilled	Hold in Stainless Steel, chilled	Hold in Stainless Steel, chilled	Stability of bulk drug product is most influenced by temperature, which is controlled similarly across all sites. Clinical manufacturing experience includes exposure of process streams to both 4.2 1st ind and stainless steel. Site-to-site shipment considered likely to be worst case as compared to static hold. All holds to be validated.
Sterile Filtration	4.2 1st ind					Membrane area scaled appropriate to batch volume. All filtration parameters for commercial production are validated.

Table 3.2.P.2.3-2. Comparison of Drug Product Manufacturing Processes (Multiple Markets)

Process Step	Clinical Supplies	Emergency Supply	Commercial Supply (Multiple Markets)			Assessment
Container Closure Components	Polymun fill/finish: Stopper: West 4.2 1st ind Vial: Nuova Ompi Puurs fill/finish: Stopper: Datwyler 4.2 1st ind Vial: Schott	Stopper: Datwyler 4.2 1st ind Vial: Schott	Stopper: Datwyler 4.2 1st ind Vial: Gerresheimer	Stopper: Datwyler 4.2 1st ind Vial: Schott, Nuova Ompi	Stopper: Datwyler 4.2 1st ind Vial: Schott, Nuova Ompi	Container closure combinations are comparable and are supported with site qualification. Similar site procedures for component preparation.
Aseptic Filling	Fill weight target varied.	Fill weight target set to achieve 0.45 mL per vial	Fill weight target set to achieve 0.45 mL per vial	Fill weight target set to achieve 0.45 mL per vial	Fill weight target set to achieve 0.45 mL per vial	Fill volume set as appropriate to target nominal amount per vial.
Visual Inspection	Automated or manual	Automated or manual	Automated or manual	Automated or manual	Automated or manual	Automated inspection technology implemented for improved process efficiency for routine commercial production.
Labeling and Freezing	Manual labeling Freezing in traditional freezers.	Automated labeling Freezing in traditional freezers.	Automated labeling Freezing in traditional freezers.	Automated labeling Freezing in traditional freezers.	Automated labeling Freezing in traditional freezers.	Automation implemented for increased process efficiency.
Storage, Packaging and Shipment	-80 to -60 °C Smaller clinical packaging appropriate to trials.	-90 to -60 °C Larger corrugated boxes for packaging of vials.	-90 to -60 °C Larger corrugated or paperboard boxes for packaging of vials.	-90 to -60 °C Larger corrugated or paperboard boxes for packaging of vials.	-90 to -60 °C Larger corrugated or paperboard boxes for packaging of vials.	Same target temperature across all sites, minor changes in range depending on equipment. Multiple box configurations implemented for distribution.

a. Croda was originally a sub-contractor to Avanti and as of August 2020 has merged with Avanti.

Abbreviations: FC = Flexible Containers; 4.2 1st ind BDP = bulk drug product; TFF = Tangential Flow Filtration

3.2.P.2.3.1.2. Comparability of Product Quality

The assessment of product quality comparability will take a step-wise approach. As a first step, comparability is demonstrated in this section between clinical and emergency supply drug product lots through a combination of release and heightened characterization testing. Subsequently, comparability will be established among representative lots of commercial drug product produced from each of the commercial manufacturing sites (commercial manufacturing sites applicable to this dossier are specified in [Section 3.2.P.3.1 Manufacturer\(s\)](#)) through completion of PPQ and a continued process verification program.

Initial comparability data for “classical” versus “upscale” process materials are presented in [Section 3.2.P.2.3 Process Development and Characterization](#), including data for particle size, polydispersity, encapsulation efficiency, N/P ratio, SAXS, and biological activity in a mouse immunogenicity model.

Results of release testing presented in [Table 3.2.P.2.3-3](#) further demonstrate consistent product quality among recently-manufactured lots (EE8492, EE8493, EJ0553, EJ1685, EJ1686 and EK1768), supporting the consistency of the “upscale” process and further demonstrating the capability of the “scale out” approach of additional T-mixers for added process capacity and throughput.

As noted in [Table 3.2.P.2.3-2](#), comparability of ALC-0315, DSPC and cholesterol from different vendors will be established at the raw material level and with lab scale LNP assessment. An initial assessment of lipid comparability can be made from drug product lots manufactured using ALC-0315 from different vendors (see [Section 3.2.P.2.3 Lot Genealogy and Usage](#) for a table of lipid suppliers used in each DP lot). There is no change in ALC-0159 supplier.

Table 3.2.P.2.3-3. BNT162b2 Comparability of Release Test Results

Manufacturing Information								
Lot #	Clinical ^a	Clinical ^b	EE8492	EE8493	EJ0553	EJ1685	EJ1686	EK1768
Drug Substance Manufacturing Site	BioNTech	BioNTech	Pfizer, Andover	Pfizer, Andover	Pfizer, Andover	BioNTech; Rentschler	BioNTech; Rentschler	Pfizer, Andover
DS Batch Scale (L)	4.2 1st ind							
DS Process	Process 1	Process 1	Process 2	Process 2	Process 2	Process 2	Process 2	Process 2
DP LNP Manufacturing Site	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun
DP Fill/Finish Manufacturing Site	Polymun	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs
DP Fill/Finish DOM	Apr -Jul 2020	Jul 2020	05-Aug-2020	05-Aug-2020	25-Sep-2020	05-Oct-2020	07-Oct-2020	16-Oct-2020
Drug Product Analytical Information								
Release Test	Acceptance Criteria	Clinical Range	Results					
Appearance	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension
Appearance (Visible Particles)	May contain white to off-white opaque, amorphous particles	Free from observable particles	Essentially free from visible particulates	Essentially free from visible particulates	Essentially free from visible particulates	Essentially free from visible particulates	Essentially free from visible particulates	Essentially free from visible particulates
Subvisible Particles	4.2 1st ind							
pH	6.9 – 7.9	7.1-7.2	7.1	7.1	7.2	7.2	7.2	7.2
Osmolality	4.2 1st ind							
LNP Size	4.2 1st ind							
LNP Polydispersity	4.2 1st ind							
RNA Encapsulation	4.2 1st ind							
RNA Content	4.2 1st ind							
ALC-0315 Content	4.2 1st ind							

Table 3.2.P.2.3-3. BNT162b2 Comparability of Release Test Results

Manufacturing Information								
Lot #	Clinical ^a	Clinical ^b	EE8492	EE8493	EJ0553	EJ1685	EJ1686	EK1768
Drug Substance Manufacturing Site	BioNTech	BioNTech	Pfizer, Andover	Pfizer, Andover	Pfizer, Andover	BioNTech; Rentschler	BioNTech; Rentschler	Pfizer, Andover
DS Batch Scale (L)	4.2 1st ind							
DS Process	Process 1	Process 1	Process 2	Process 2	Process 2	Process 2	Process 2	Process 2
DP LNP Manufacturing Site	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun
DP Fill/Finish Manufacturing Site	Polymun	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs
DP Fill/Finish DOM	Apr -Jul 2020	Jul 2020	05-Aug-2020	05-Aug-2020	25-Sep-2020	05-Oct-2020	07-Oct-2020	16-Oct-2020
Drug Product Analytical Information								
Release Test	Acceptance Criteria	Clinical Range	Results					
ALC-0159 Content	4.2 1st ind							
DSPC Content	4.2 1st ind							
Cholesterol Content	4.2 1st ind							
Container Content for Injections	Not less than the sum of the nominal values of 5 doses	NA	Not less than the sum of the nominal values of 5 doses.	Not less than the sum of the nominal values of 5 doses.	Not less than the sum of the nominal values of 5 doses.	Not less than the sum of the nominal values of 5 doses.	Not less than the sum of the nominal values of 5 doses.	Not less than the sum of the nominal values of 5 doses.
Lipid Identities	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	Conforms to reference	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)
Identity of Encoded RNA Sequence	Identity confirmed	NA	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed
In Vitro Expression	4.2 1st ind							
RNA Integrity	4.2 1st ind							
Bacterial Endotoxins	4.2 1st ind							

Table 3.2.P.2.3-3. BNT162b2 Comparability of Release Test Results

Manufacturing Information								
Lot #	Clinical ^a	Clinical ^b	EE8492	EE8493	EJ0553	EJ1685	EJ1686	EK1768
Drug Substance Manufacturing Site	BioNTech	BioNTech	Pfizer, Andover	Pfizer, Andover	Pfizer, Andover	BioNTech; Rentschler	BioNTech; Rentschler	Pfizer, Andover
DS Batch Scale (L)	4.2 1st ind							
DS Process	Process 1	Process 1	Process 2	Process 2	Process 2	Process 2	Process 2	Process 2
DP LNP Manufacturing Site	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun	Polymun
DP Fill/Finish Manufacturing Site	Polymun	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs	Pfizer, Puurs
DP Fill/Finish DOM	Apr -Jul 2020	Jul 2020	05-Aug-2020	05-Aug-2020	25-Sep-2020	05-Oct-2020	07-Oct-2020	16-Oct-2020
Drug Product Analytical Information								
Release Test	Acceptance Criteria	Clinical Range	Results					
Sterility	No growth detected	Sterile	No growth detected	No growth detected	No growth detected	No growth detected	No growth detected	No growth detected

- a. Clinical lots BCV40420-A, BCV40620-A, BCV40620-B, BCV40620-C, BCV40620-D, BCV40720-A, BCV40720-B, BCV40720-C
b. Clinical lots BCV40720-P and BCV40820-P
c. Data not available (NA) at the time of filing.

The BNT162b2 drug product comparability assessment presented here includes drug product lots used in Phase 1, Phase 2, and Phase 3 clinical studies, as well as a drug product lot manufactured for emergency supply in the US market ([Table 3.2.P.2.3-4](#)). The study includes representative lots from each clinical and emergency supply manufacture campaign, representing both the Polymun, Austria and Puurs, Belgium drug product fill sites ([Table 3.2.P.2.3-1](#)).

The panel of tests, both release and heightened characterization, performed to evaluate drug product comparability are shown in [Table 3.2.P.2.3-5](#). The tests executed to support the comparability evaluation are described in [Section 3.2.S.4.2 Analytical Procedures](#), [Section 3.2.S.2.6 Analytical Method Evolution](#), [Section 3.2.P.2.3 Analytical Method Evolution](#), and [Section 3.2.P.5.2 Analytical Procedures](#).

Additional heightened characterization methods were performed to evaluate selected drug product LNP and purity attributes. Comparability results are tabulated in [Table 3.2.P.2.3-6](#) and discussed in [Section 3.2.P.2.3.1.2.1](#). Supportive profiles from selected heightened characterization methods are provided in [Section 3.2.P.2.3.1.2.2](#).

Table 3.2.P.2.3-4. BNT162b2 Clinical and Emergency Supply^a Drug Product Lots

DP Lot Number ^b	Purpose of Material	Included in Comparability Evaluation	Drug Substance Batch(es)
BCV40420-A	Clinical, Stability	X	R427-P020.2-DS
BCV40620-A	Clinical, Stability	X	R438-P020.2-DS
BCV40620-B	Clinical		
BCV40620-C	Clinical		
BCV40620-D	Clinical	X	
BCV40720-A	Clinical, Stability	X	R443-P020.2-DS
BCV40720-B	Clinical		
BCV40720-C	Clinical, Stability		
BCV40720-P	Clinical, Stability	X	
BCV40820-P	Clinical Inventory, Stability	X	R445-P020.2-DS
EE8492	Emergency supply ^a , Stability		20Y513C101
EE8493	Clinical Inventory, Emergency supply ^a , Stability	X	
EJ0553	Emergency supply ^a , Clinical inventory, Stability		20Y513C501
EJ1685	Emergency supply ^a , Clinical inventory, Stability		20E162001 (1071539)
EJ1686	Emergency supply ^a , Clinical inventory		20E162001 (1071539)
EK1768	Emergency supply ^a , Clinical inventory		20Y513C401

a. Emergency supply designation applies to U.S. market.

b. See [Section 3.2.P.2.3 Lot Genealogy and Usage](#) for drug product manufacturing site and scale, date, and drug substance manufacturing site.

Table 3.2.P.2.3-5. BNT162b2 Drug Product Comparability Testing Panel

Quality Attribute	Analytical Procedure	Release / Characterization
Composition and Strength		
Appearance	Appearance (Visual)	Release
Appearance (Visible Particulates)	Appearance (Particles) ^a	Release
Subvisible Particles	Subvisible Particulate Matter ^{a, b}	Release
pH	Potentiometry ^a	Release
Osmolality	Osmometry ^{a, c}	Release
LNP Size	Dynamic Light Scattering (DLS)	Release
LNP Polydispersity	Dynamic Light Scattering (DLS)	Release
RNA Encapsulation	Fluorescence assay	Release
RNA content	Fluorescence assay	Release
ALC-0315 content	HPLC-CAD	Release
ALC-0159 content	HPLC-CAD	Release
DSPC content	HPLC-CAD	Release
Cholesterol content	HPLC-CAD	Release
Surface Charge	Zeta Potential ^c	Characterization
Size Distribution and Shape	AF4 ^c	Characterization
Surface PEG Characterization	¹ H NMR ^c	Characterization
Identity		
Lipid identities	HPLC-CAD	Release
Identity of encoded RNA sequence	RT-PCR ^{d, e}	Release
Potency		
In Vitro Expression	Cell-based Flow Cytometry ^{d, e}	Release
Purity		
RNA Integrity	Capillary Gel Electrophoresis ^{d, e}	Release
5'- Cap	RP-HPLC ^{d, e}	Characterization
Poly(A) Tail	ddPCR ^{d, e}	Characterization
Poly(A) Tail: Length and Distribution	RP-HPLC ^c	Characterization

a. Compendial

b. USP<787> (obscuration method), and aligned with upcoming (Jan 2021) revision of Ph. Eur. 2.9.19

c. USP<785>; also in accordance with Ph Eur. 2.2.35, with minor difference in instrument calibration

d. New test or updated method introduced during product development ([Section 3.2.S.2.6 Analytical Method Evolution](#) and [Section 3.2.P.2.3 Analytical Method Evolution](#)).

e. Tested side-by-side

Abbreviations: LNP = Lipid nanoparticles; CAD = charged aerosol detector; RT-PCR = reverse transcription polymerase chain reaction; AF4 = asymmetric flow field flow fractionation; ¹H NMR = Proton Nuclear Magnetic Resonance; ddPCR = droplet digital PCR; qPCR = quantitative PCR; GC = gas chromatography

Table 3.2.P.2.3-6. Comparability Data for BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Lot Number						
		BCV40420-A	BCV40620-A	BCV40620-D	BCV40720-A	BCV40720-P	BCV40820-P	EE8493
		Results						
Appearance	Appearance (Visual)	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension	White to off-white suspension
Appearance (visible particulates)	Appearance (Particles)	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles	Free from observable particles
Subvisible particles	Subvisible particulate matter	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
pH	Potentiometry	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Osmolality	Osmometry	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
LNP size	Dynamic light scattering (DLS)	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
LNP polydispersity	Dynamic light scattering (DLS)	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
RNA encapsulation	Fluorescence assay	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
RNA content	Fluorescence assay	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
ALC-0315 content	HPLC-CAD	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
ALC-0159 content	HPLC-CAD	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
DSPC content	HPLC-CAD	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Cholesterol content	HPLC-CAD	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Zeta potential (mV) ^a	Electrophoretic light scattering	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Size Distribution (Rh) (nm) ^a	AF4	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Particle shape (Rz/Rh) ^a	AF4	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind	4.2 1st ind
Lipid identities	HPLC-CAD	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference

Table 3.2.P.2.3-6. Comparability Data for BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Lot Number						
		BCV40420-A	BCV40620-A	BCV40620-D	BCV40720-A	BCV40720-P	BCV40820-P	EE8493
		Results						
Identity of encoded RNA sequence ^a	RT-PCR	Identity Confirmed	Identity Confirmed	Identity Confirmed	Identity Confirmed	Identity Confirmed	Identity Confirmed	Identity Confirmed
In vitro expression (% cells positive 150 ng) ^a	Cell-based flow cytometry	4.2.1						
RNA integrity ^a	Capillary gel electrophoresis	4.2.1						
5' - Cap ^a	RP-HPLC	4.2.1						
Capped-Intact RNA (%) ^b	Capillary gel electrophoresis and RP-HPLC	4.2.1						
Poly (A) Tail ^a	ddPCR	4.2.1						
Poly A Tail: Length and distribution (%) ^a	RP-HPLC	4.2 1st ind						

a. Tested side-by-side

b. Capped Intact RNA (%) = RNA Integrity (%) x 5'-Cap

Abbreviations: LNP = Lipid nanoparticles; CAD = charged aerosol detector; RT-PCR = reverse transcription polymerase chain reaction; AF4 = asymmetric flow field flow fractionation; NMR = Nuclear Magnetic Resonance; ddPCR = droplet digital PCR; qPCR = quantitative PCR; RP-HPLC = reversed-phase high performance liquid chromatography; GC = gas chromatography; Rh = hydrodynamic radius; Rz = root mean square radius.

3.2.P.2.3.1.2.1. Discussion of Release and Characterization Data to Support BNT162b2 Product Quality Comparability

Release and characterization data supporting the BNT162b2 drug product comparability assessment are summarized in Table 3.2.P.2.3-6. All quality attributes evaluated during drug product release testing (noted in Table 3.2.P.2.3-5) met the specification acceptance criteria at the time of testing (Section 3.2.P.5.4 Batch Analyses) and are consistent across clinical and emergency supply lots. Subvisible particles (4.2 1st ind) were higher in lot EE8493, but remain well below compendial limits. Subvisible particle content will continue to be monitored for future DP lots. (4.2 1st ind) in drug product lot EE8493; however, when (4.2 1st ind) drug product load was assessed, the in vitro expression from lot EE8493 (4.2 1st ind) was within the range observed in clinical lots (4.2 1st ind).

Slightly lower RNA integrity was observed in lot EE8493; however, the side-by-side testing result for this lot (4.2 1st ind) is consistent with the RNA integrity of the starting DS batch 20Y513C101 (4.2 1st ind) as measured in the side-by-side DS comparability assessment (Section 3.2.S.2.6 Development History and Comparability Assessment). Additional characterization testing revealed a slightly higher relative abundance of 5'-capped RNA in lot EE8493 (4.2 1st ind) again, consistent with the starting DS batch level of (4.2 1st ind), as measured in the side-by-side DS comparability assessment. As RNA integrity and 5'-cap attributes are critical to translation of the protein antigen in vivo, the proportion of capped-intact RNA is used to compare the clinical and emergency supply lots that were tested side-by-side (Table 3.2.P.2.3-6). The range of capped-intact RNA in clinical lots is (4.2 1st ind) as compared to (4.2 1st ind) for the emergency supply lot EE8493. The proportion of poly-adenylated RNA as determined using ddPCR is (4.2 1st ind) for all DP lots evaluated. Small differences in poly(A) content, (4.2 1st ind) are attributed to method variability, as it is not practically feasible to achieve greater than (4.2 1st ind) poly-adenylated RNA content. In addition, heightened characterization results for poly(A) tail length and distribution (discussed below and quantified in Table 3.2.P.2.3-6) are comparable across all lots evaluated. Overall, small differences the clinical and emergency supply lots are therefore not expected to impact efficacy.

Heightened characterization of the drug product LNP enabled comparative assessment of surface charge by zeta potential measurements, size distribution by AF4, and surface PEG characterization by ¹H NMR. These methods are described further in Section 3.2.P.2.2 Drug Product. Quantitative results from zeta potential and AF4 measurements are provided in Table 3.2.P.2.3-6. All drug product lots demonstrated comparable surface charge with nearly neutral zeta potential. Size distribution by AF4 provides direct measurement of polydispersity, and all lots showed a similar hydrodynamic radius range. AF4 also provides shape information, and the Rz/Rh ratio is consistent for all tested drug product lots. ¹H NMR of drug product LNP provides information on surface PEG, and the spectra are comparable for all batches. ¹H NMR spectral data are provided below in Section 3.2.P.2.3.1.2.2.1.

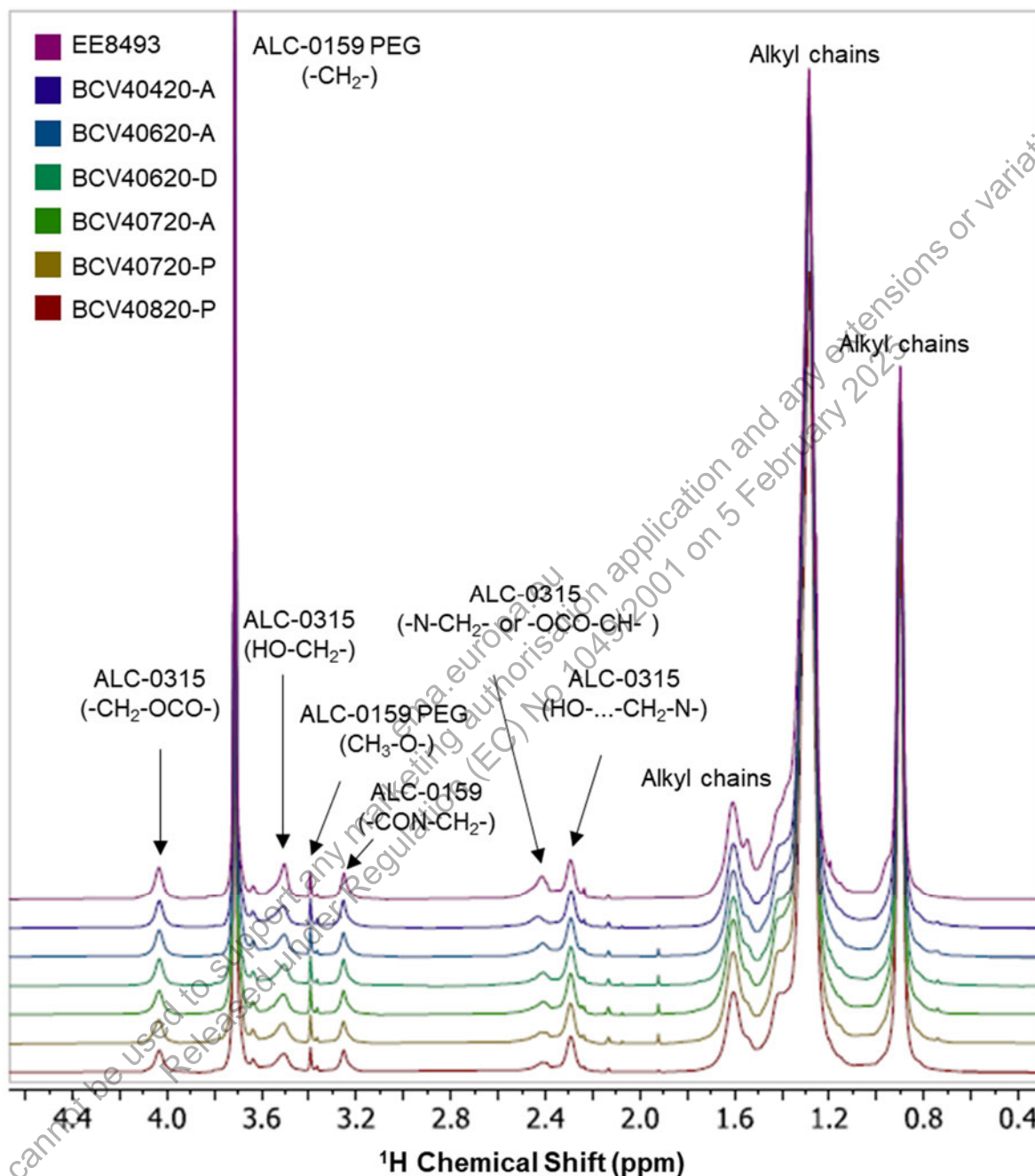
3.2.P.2.3.1.2.2. Additional Heightened Characterization Profiles to Support BNT162b2 Product Quality Comparability

Supportive data profiles are provided below for side-by-side heightened characterization testing of BNT162b2 drug product lots. ^1H NMR was used to characterize surface PEG as part of this comparability assessment. Additionally, heightened characterization of the extracted RNA enabled comparative chromatographic assessment of the 5'-cap and poly(A) tail attributes.

3.2.P.2.3.1.2.2.1. BNT162b2 Drug Product Surface PEG Characterization by ^1H NMR

All the drug product lots were subjected to 1D proton NMR analysis in 1x PBS pH 7.4, 10% D_2O . The spectra are shown in [Figure 3.2.P.2.3-1](#) and all the lots have visually superimposable spectra. Major peaks are labeled for assigned protons (see [Section 3.2.P.2.2 Drug Product](#) for detailed peak characterization).

Figure 3.2.P.2.3-1. ^1H NMR Spectra for BNT162b2 Drug Product Lots



3.2.P.2.3.1.2.2.2. Comparative Analysis of BNT162b2 Drug Product 5'-Cap by RP-HPLC

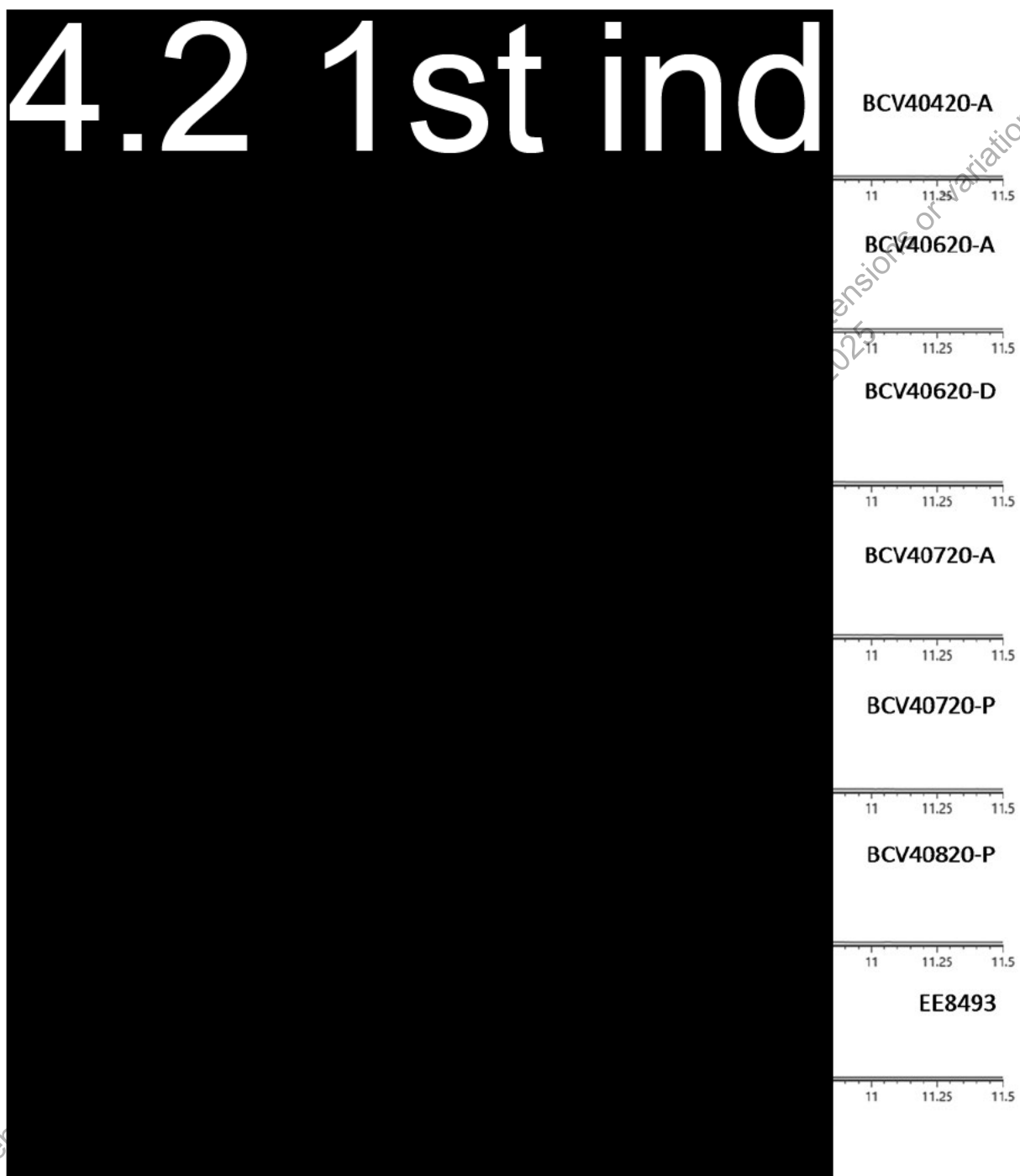
The characterization of the 5' end capped (5'-Cap) and un-capped species of BNT162b2 DP was accomplished by ion-pair reversed-phase high performance liquid chromatography (RP-HPLC). Sample handling and chromatography follow the method described in [Section 3.2.S.4.2 Reversed Phase – High Performance Liquid Chromatography \(RP-HPLC\)](#).

The identification of capped and un-capped species by mass spectrometry are presented in Section 3.2.S.3.1.3 5'-Cap Characterization by LC-UV/MS (in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#)).

Using LC-UV and peak integration, the 5'-cap levels for all DP lots exceed 4.2 1st ind (Table 3.2.P.2.3-6), with slightly higher levels of 5'-cap observed in lot EE8493 (Figure 3.2.P.2.3-2). Lot EE8493 derives from drug substance batch 20Y513C101, which shows similar levels of 5'-capped RNA (Section 3.2.S.4.4 Batch Analyses). Heightened mass spectrometric characterization of the 5'-cap species in drug substance batch 20Y513C101 was conducted as part of the drug substance comparability evaluation (Section 3.2.S.2.6 Development History and Comparability Assessment), and confirms that the same 5'-capped and uncapped species are present in Process 1 and Process 2 drug substance batches. Consistent with this observation, the drug product chromatographic profiles shown in Figure 3.2.P.2.3-2 demonstrate that no new peaks are observed in emergency supply lot EE8493 or any of the clinical trial material lots.

It is anticipated that a higher 5'-cap level has the potential to contribute to improved stability and efficacy of the drug product. Therefore, the drug product lots are considered comparable with respect to 5'-cap.

Figure 3.2.P.2.3-2. 5'-Cap RP-HPLC Chromatograms for BNT162b2 Drug Product

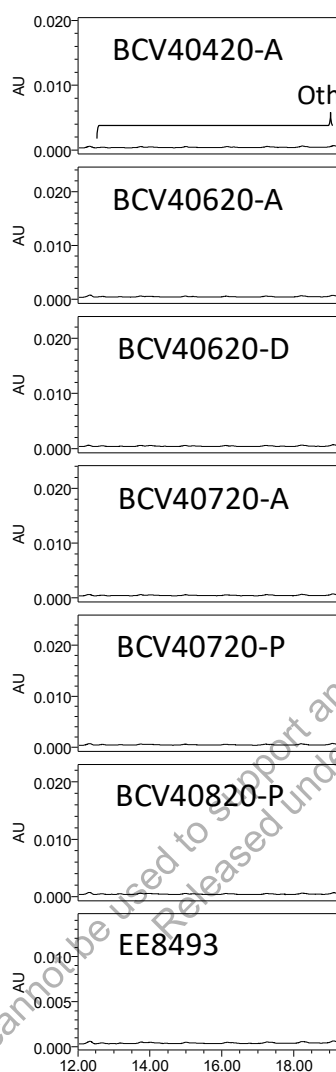


3.2.P.2.3.1.2.2.3. Poly(A) Tail Length and Distribution by RP-HPLC

To evaluate the distribution of the poly(A) tail segments of ~30 adenosine nucleotides (A30) and ~70 adenosine nucleotides (L70), RNA was extracted from BNT162b2 drug product lots and subsequently digested with RNase T1 and RNase A. The prepared samples were analyzed by ion-pair reversed-phase HPLC (RP-HPLC). [Figure 3.2.P.2.3-3](#) shows the

BNT162b2 drug product poly(A) tail chromatographic profiles. Visual assessment of the chromatograms demonstrates comparable overall distributions of A30 and L70 species, with a slight broadening of the L70 peak in lot EE8493, which is consistent with the profile of the parent DS batch 20Y513C101 ([Section 3.2.S.2.6 Development History and Comparability Assessment](#)). Quantitative assessment ([Table 3.2.P.2.3-6](#)) further demonstrates comparable relative A30, L70, and Other species content.

Figure 3.2.P.2.3-3. Poly(A) Tail RP-HPLC Chromatograms for BNT162b2 Drug Product Lots



4.2 1st ind

3.2.P.2.3.1.3. Overall Conclusions for Comparability

The comparability assessment presented here focused on an assessment of the process designs ([Section 3.2.P.2.3.1.1](#)) and an evaluation of the product quality in clinical and

emergency supply lots ([Section 3.2.P.2.3.1.2](#)). Comparability of the “classical” and “upscale” LNP processes is evaluated in [Section 3.2.P.2.3 Process Development and Characterization](#) and is further demonstrated in the comparison of clinical supply lots (“classical” LNP process) and a representative emergency supply lot (“upscale” LNP process).

The BNT162b2 drug product analytical comparability evaluation employed release testing and heightened characterization methods to evaluate product quality in drug product lots manufactured using the Polymun, Austria and Puurs, Belgium drug product fill sites. Representative lots from each manufacturing campaign supporting clinical trials and emergency supply use were included in the study. Release data and heightened analytical characterization methods demonstrate that the drug product lots evaluated in the current study are considered comparable, with only small differences that are not expected to impact efficacy.

A comprehensive demonstration of comparability among clinical supplies and commercial product including an assessment of the starting drug substance batches, raw materials (e.g. ALC-0315, DSPC and cholesterol) from different vendors, process designs and comprehensive characterization of the resulting product quality is planned.