EMA/CHMP/448917/2021
Committee for Medicinal Products for Human Use (CHMP)

Type II group of variations assessment report

Procedure No. EMEA/H/C/005735/II/0056/G

Invented name: COMIRNATY

International non-proprietary name: tozinameran

Marketing authorisation holder (MAH): BioNTech Manufacturing GmbH

This application is in the area of: Quality

eCTD sequences related to the procedure: 0144, 0224, 0279

Status of this report and steps taken for the assessment				
Current step	**Description**	**Planned date**	**Actual Date**	**Need for discussion**
☐	Start of procedure	16 Aug 2021	16 Aug 2021	☐
☐	CHMP Rapporteur Assessment Report	20 Sep 2021	21 Sep 2021	☐
☐	CHMP members comments	04 Oct 2021	04 Oct 2021	☐
☐	Updated CHMP Rapporteur Assessment Report	07 Oct 2021	07 Oct 2021	☐
☐	Request for Supplementary Information	14 Oct 2021	14 Oct 2021	☐
☐	Submission of responses	16 Nov 2021	05 Nov 2021	☐
☐	Re-start of procedure	17 Nov 2021	17 Nov 2021	☐
☐	CHMP Rapporteur Assessment Report	01 Dec 2021	01 Dec 2021	☐
☐	CHMP members comments	06 Dec 2021	06 Dec 2021	☐
☐	Updated CHMP Rapporteur Assessment Report	09 Dec 2021	10 Dec 2021	☐
☐	2nd Request for Supplementary Information	16 Dec 2021	16 Dec 2021	☐
☐	Submission of responses	22 Dec 2021	22 Dec 2021	☐
☐	Re-start of procedure	29 Dec 2021	29 Dec 2021	☐
☐	CHMP Rapporteur Assessment Report	02 Feb 2022	03 Feb 2022	☐
☐	CHMP members comments	07 Feb 2022		☐
☐	Updated CHMP Rapporteur Assessment Report	10 Feb 2022	10 Feb 2022	☐
☒	CHMP Opinion	17 Feb 2022	17 Feb 2022	☐

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Declarations

This application includes an Active Substance Master File (ASMF):

☐Yes ☒ No

☒The assessor confirms that proprietary information on, or reference to, third parties (e.g. ASMF holder) or products are not included in this assessment, including in the Product Information, if any, unless there are previous contracts and/or agreements with the third party(ies).

Whenever the above box is un-ticked please indicate section and page where confidential information is located here:

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1. Background information on the procedure

Pursuant to Article 7.2 of Commission Regulation (EC) No 1234/2008, BioNTech Manufacturing GmbH submitted to the European Medicines Agency on 2 August 2021 an application for a group of variations.

The following changes were proposed:

Variations requested		**Type**	**Annexes affected**
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.I.z	B.I.z - Quality change - Active substance - Other variation	Type IB	None
C.I.11.b	C.I.11.b - Introduction of, or change(s) to, the obligations and conditions of a marketing authorisation, including the RMP - Implementation of change(s) which require to be further substantiated by new additional data to be submitted by the MAH where significant assessment is required	Type II	IIE
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.I.b.1.a	B.I.b.1.a - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.I.b.1.c	B.I.b.1.c - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent - Addition of a new specification parameter to the specification with its corresponding test method	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification	Type IB	None

	limits for medicinal products subject to OCABR		
B.I.d.1.c	B.I.d.1.c - Stability of AS - Change in the re-test period/storage period or storage conditions - Change to an approved stability protocol	Type IA	None
C.I.11.b	C.I.11.b - Introduction of, or change(s) to, the obligations and conditions of a marketing authorisation, including the RMP - Implementation of change(s) which require to be further substantiated by new additional data to be submitted by the MAH where significant assessment is required	Type II	IIE
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None

C.I.11.b, (Type II)- To submit additional data to complete characterisation of the active substance and finished product, which are a condition to the Marketing Authorisation (Special Obligation SO1)

C.I.11.b, (Type II)- To submit additional data to enhance the control strategy, including the active substance and finished product specifications, which are a condition to the Marketing Authorisation (Special Obligation SO2)

B.I.b.1.a, (Type IB)- To tighten the active substance specification limits for release and stability testing for 5'-Cap from [redacted]

B.I.b.1.c, (Type IB)- To add poly(A) tail length to the specifications of the active substance (together with its corresponding test method Ion pair-reverse phase-HPLC, IP-RP-HPLC). The acceptance criterion is "Poly(A) Tail Length Confirmed".

B.II.d.1.b, (Type IB) To tighten the finished product specification limits for Osmolality from [redacted] mOsmol/kg to [redacted] mOsmol/kg

B.II.d.1.b, (Type IB)To tighten the finished product specification limits for Lipid nanoparticles (LNP) size for release from 40-120 nm to 56-101 nm and for stability from 40-120 nm to 56-120 nm.

B.II.d.1.b, (Type IB)To tighten the finished product specification limits for RNA encapsulation from [redacted]

B.II.d.1.b, (Type IB) To tighten the finished product specification limits for RNA content from [redacted] mg/mL to [redacted] mg/mL.

B.II.d.1.b, (Type IB) To tighten the finished product specification limits for ALC-0315 content from [redacted] mg/mL to [redacted] mg/mL.

B.II.d.1.b, (Type IB) To tighten the finished product specification limits for ALC-0159 content from [redacted] mg/mL to [redacted] mg/mL.

B.II.d.1.b, (Type IB) To tighten the finished product specification limits for DSPC content from [redacted] mg/mL to [redacted] mg/mL.

B.II.d.1.b, (Type IB) To tighten the finished product specification limits for cholesterol content from [redacted]

[redacted] mg/mL to [redacted] mg/mL.

B.I.d.1.c, (Type IA) Changes to the approved stability protocol of the active substance to tighten the acceptance criteria for RNA integrity for a few drug substance stability batches.

B.I.z, (Type IB) Substantial updates to Module 3.2.S, to correct typographical errors in a stability study acceptance criteria and in stability results for a few drug product lots.

These variations also address recommendations relating to the in-vitro expression (IVE) assay (REC10), additional finished product stability data (REC20) and statistical evaluation of active substance stability data (REC VAR IB-01).

2. Overall conclusion and impact on the benefit/risk balance

To address specific obligations on quality, the MA Holder provided relevant data and proposed changes to the dossier, in line with the requirements and due dates set by CHMP.

With regards to SO1, the MA Holder has provided additional characterisation data as requested.

a) The potential for truncated transcripts to produce proteins/peptides was further investigated using a cell-free in vitro expression system. No truncated or other protein species were detected beyond the background bands observed in the negative control sample. The MAH will complement the characterization exercise using the cell-free in vitro translation system with additional tozinameran batches.

b) It is sufficiently demonstrated that the major proportion of fragmented species contains the 5'-cap but lacks the poly(A) tail.

c) WB results obtained by three different antibodies, specific for the S1 domain, the receptor binding domain and the S2 domain, respectively, were presented and compared to theoretical masses of the S-protein and the subdomains in glycosylated and non-glycosylated forms. It is sufficiently justified that the major band monitored corresponds to the heavily glycosylated S-protein.

With regards to SO2, the MA Holder provided additional information to enhance the control strategy, including the active substance and finished product specifications.

a) For active substance the acceptance criterion for 5'-Cap has been tightened. For finished product acceptance criteria for osmolality, LNP size, RNA encapsulation, RNA content and lipids content (ALC-0315, ALC-0159, DSPC, cholesterol) and *in vitro* expression (IVE) assay have been tightened. The revised limits are sufficiently justified and found acceptable. The acceptance criteria for RNA integrity test in active substance and finished product specifications have been previously tightened during VAR IB-31-G.

b) A new IP-RP-HPLC has been introduced and acceptably validated.

c) The MAH previously presented data supporting the suitability of the ddPCR method, as being capable of detecting changes in the poly(A) tail content. The accuracy of the ddPCR method is sufficiently addressed.

d) The acceptance criteria for RNA integrity test in active substance and finished product specifications have been previously tightened during VAR IB-31-G. The justifications for no further tightening of the finished product specifications for RNA integrity and LNP polydispersity in the current variation have been acceptably justified.

e) Additional details have been provided to support the suitability of the IVE assay (cell-based flow cytometry) used for potency determination.

f) Evaluation of lipid-related impurities in the finished product and discussion on ALC-0315 impact on late migrating species (LMS) and RNA integrity were provided and assessed via parallel VAR II-54-G.

There were two RSIs raised during the procedure. The applicant has sufficiently answered all questions raised. Of particular note is the tightening of multiple specification parameters in previous and in this current variation application.

For the *in vitro* expression (IVE) assay, additional data from batches used in the clinical trials have been provided with a range of spike protein expression between [redacted] Consequently, the MA Holder proposes to tighten the acceptance criterion for the in vitro expression (IVE) test from "[redacted] Cells Positive" to "[redacted] Cells Positive". The approach is endorsed, and the newly proposed limit is considered clinically justified. Relevant modules of the dossier have been updated.

Based on the provided information, SO1 and SO2 are considered fulfilled. The provided data also fulfils recommendations on analytical methods (REC 10) and additional stability (REC 20).

The variation is approvable. The benefit-risk balance of COMIRNATY, remains positive.

3. Recommendations

Based on the review of the submitted data, this application regarding the following changes:

Variations requested		**Type**	**Annexes affected**
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.I.z	B.I.z - Quality change - Active substance - Other variation	Type IB	None
C.I.11.b	C.I.11.b - Introduction of, or change(s) to, the obligations and conditions of a marketing authorisation, including the RMP - Implementation of change(s) which require to be further substantiated by new additional data to be submitted by the MAH where significant assessment is required	Type II	IIE
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.I.b.1.a	B.I.b.1.a - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent - Tightening of specification limits for medicinal products	Type IB	None

	subject to OCABR		
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.I.b.1.c	B.I.b.1.c - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent - Addition of a new specification parameter to the specification with its corresponding test method	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None
B.I.d.1.c	B.I.d.1.c - Stability of AS - Change in the re-test period/storage period or storage conditions - Change to an approved stability protocol	Type IA	None
C.I.11.b	C.I.11.b - Introduction of, or change(s) to, the obligations and conditions of a marketing authorisation, including the RMP - Implementation of change(s) which require to be further substantiated by new additional data to be submitted by the MAH where significant assessment is required	Type II	IIE
B.II.d.1.b	B.II.d.1.b - Change in the specification parameters and/or limits of the finished product - Tightening of specification limits for medicinal products subject to OCABR	Type IB	None

☒ is recommended for approval.

Amendments to the marketing authorisation

In view of the data submitted with the group of variations, amendments to Annex II are recommended.

The following obligations have been fulfilled, and therefore it is recommended that they be deleted from the Annex II to the Opinion:

- SO1: In order to ensure consistent product quality, the MAH should provide additional information to enhance the control strategy, including the active substance and finished product specifications.
- SO2: In order to ensure consistent product quality, the MAH should provide additional information to enhance the control strategy, including the active substance and finished product specifications.

4. EPAR changes

The table in Module 8b of the EPAR will be updated as follows:

Scope

Please refer to the Recommendations section above.

Summary

To address specific obligations on quality, the MA Holder provided relevant data and proposed changes to the dossier, in line with the requirements and due dates set by CHMP.

With regards to SO1, the MA Holder has provided additional characterisation data as requested.

a) The potential for truncated transcripts to produce proteins/peptides was further investigated using a cell-free in vitro expression system. No truncated or other protein species were detected beyond the background bands observed in the negative control sample. The MAH will complement the characterization exercise using the cell-free in vitro translation system with additional tozinameran batches.

b) It is sufficiently demonstrated that the major proportion of fragmented species contains the 5'-cap but lacks the poly(A) tail.

c) WB results obtained by three different antibodies, specific for the S1 domain, the receptor binding domain and the S2 domain, respectively, were presented and compared to theoretical masses of the S-protein and the subdomains in glycosylated and non-glycosylated forms. It is sufficiently justified that the major band monitored corresponds to the heavily glycosylated S-protein.

With regards to SO2, the MA Holder provided additional information to enhance the control strategy, including the active substance and finished product specifications.

a) For active substance the acceptance criterion for 5'-Cap has been tightened. For finished product acceptance criteria for osmolality, LNP size, RNA encapsulation, RNA content and lipids content (ALC-0315, ALC-0159, DSPC, cholesterol) and *in vitro* expression (IVE) assay have been tightened. The revised limits are sufficiently justified and found acceptable. The acceptance criteria for RNA integrity test in active substance and finished product specifications have been previously tightened during VAR IB-31-G.

b) A new test method has been introduced and acceptably validated.

c) The MAH previously presented data supporting the suitability of the ddPCR method, as being capable of detecting changes in the poly(A) tail content. The accuracy of the ddPCR method is sufficiently addressed.

d) The acceptance criteria for RNA integrity test in active substance and finished product specifications have been previously tightened during VAR IB-31-G. The justifications for no further tightening of the finished product specifications for RNA integrity and LNP polydispersity in the current variation have been acceptably justified.

e) Additional details have been provided to support the suitability of the IVE assay (cell-based flow cytometry) used for potency determination.

f) Evaluation of lipid-related impurities in the finished product and discussion on ALC-0315 impact on late migrating species (LMS) and RNA integrity were provided and assessed via parallel VAR II-54-G.

Based on the provided information, SO1 and SO2 are considered fulfilled. The provided data also fulfils recommendations on analytical methods (REC 10) and additional stability (REC 20).

The Annex IIE has been updated as follows:

- SO1 (relating to characterisation of the active substance and finished product) is deleted from the list of specific obligations.
- SO2 (relating to control strategy, including the active substance and finished product specifications) is deleted from the list of specific obligations.

The information after this line is considered commercially confidential and may not be disclosed to third parties in accordance with the 'HMA/EMA guidance on the identification of commercially confidential information and personal data'.

Annex: Rapporteur's assessment comments on the type II variation

5. Introduction

The primary purpose of this submission is to fulfil Specific Obligations 1 and 2 (SO1 and SO2). To fulfill SO1, additional characterisation information for the active substance and finished product is provided. To fulfil SO2, the drug substance and drug product release and stability specification acceptance criteria have been reassessed and revised, as appropriate.

Along with fulfilment of SO2 part f in EMEA/H/C/005735/II/0054/G, SO2 is now completely fulfilled. Updated drug substance and drug product stability data (including additional batches/lots and data at additional stability study time points) are provided in support of the updated specifications. Additionally, ion pair-reverse phase-HPLC (IP-RPHPLC) has been introduced as a new drug substance release method to measure the length of the poly(A) tail.

The Applicant is submitting a grouping of variations to support:

- Two Type II variation (C.I.11.b), Implementation of changes and provision of data to fulfill SO1 and SO2.
- A Type IB variation (B.I.b.1.a), Change in the specification parameters and/or limits of an active substance, starting material/intermediate/reagent used in the manufacturing process of the active substance, Tightening of specification limits for medicinal products subject to Official Control Authority Batch Release, to tighten the drug substance release and stability acceptance criterion for 5'-Cap (condition 1 unmet).
- A Type IB variation (B.I.b.1.c), Change in the specification parameters and/or limits of an active substance, starting material/intermediate/reagent used in the manufacturing process of the active substance, Addition of a new specification parameter to the specification with its corresponding test method, to add the IP-RPHPLC method for poly(A) tail length and an associated acceptance criterion for drug substance release (condition 1 unmet).
- A Type IB variation (B.II.d.1.b), Change in the specification parameters and/or limits of the finished product, Tightening of specification limits for medicinal products subject to Official Control Authority Batch Release, to tighten the drug substance release and stability acceptance criteria for osmolality, LNP size, RNA encapsulation, RNA content, ALC-0315 content, ALC-0159 content, DSPC content, and cholesterol content (condition 1 unmet).
- A Type IA variation (B.I.d.1.c), Change in the retest period/storage period or storage conditions of the active substance where no Ph. Eur. Certificate of Suitability covering the retest period is part of the approved dossier, Change to an approved stability protocol, to revise the stability protocol with tightened acceptance criteria for RNA integrity for a few drug substance stability batches.
- A Type IB variation (B.I.z), Editorial correction to correct typographical errors in a stability study acceptance criteria and in stability results for a few drug product lots.

Assessor´s comments

The applicant has provided an acceptable background and overview to this grouped Type II variation to fulfil specific obligations 1 and 2 (SO1 and SO2).

It is described that additional characterisation information is provided for the active substance and finished product to fulfil SO1. Furthermore, the release and stability specifications for both DS and DP have been reassessed and revised to fulfil SO2.

A present and proposed table has been provided in module 1.

6. Quality aspects

6.1. Drug substance

6.1.1. Characterisation – SO1

In order to complete the characterisation of the active substance and finished product, the MAH should provide additional data. The following data are requested in order to complete the information on the active substance and finished product characterisation.

a) Additional data is to be provided to further characterise the truncated and modified mRNA species present in the finished product both from process 1 and 2. Data are expected to cover batches used in clinical trials (for which the characterisation data could be available earlier) and the PPQ batches. These data should address results from ion pairing RP-HPLC addressing 5'cap levels and presence of A30 and L70 in the poly(A) tail. These data should further address the potential for translation into truncated S1S2 proteins/peptides or other proteins/peptides. Relevant protein/peptide characterization data for predominant species should be provided. Any homology between translated proteins (other than the spike protein) and human proteins that may, due to molecular mimicry, potentially cause an autoimmune process should be evaluated. Due date: July 2021. Interim reports: March 2021, and on a monthly basis.

b) The analysis of the main peak of the RNA integrity test representing the full-length RNA, should be also undertaken using the ion pairing RP-HPLC addressing 5'cap levels and presence of A30 and L70 in the poly (A) tail. Due date: July 2021. Interim report: March 2021

c) Additional data for the active substance are to be provided to confirm the identities of the observed Western Blot (WB) bands obtained by the in vitro expression assay. Protein heterogeneity, resulting in broad bands on the WB and uncertainties in the theoretical intact molecular weight of the spike protein, is assumed to be due to glycosylation. Therefore, to further confirm protein identities, enzymatic deglycosylation of the expressed proteins followed by WB analysis should be performed. Correlation with the calculated molecular weights of the intact S1S2 protein should be demonstrated. Due date: July 2021. Interim report: March 202

Response to a and b

The BNT162b2 fragment profile, as measured using the Fragment Analyzer CGE method, was demonstrated to be consistent between Process 1 and Process 2 drug substance (DS) in the previously submitted Response Q002 – Quality – Major Objection 04-Dec-2020 (seq 0003). The associated safety assessment revealed that the likelihood of fragmented transcripts being expressed into proteins is low, with a rationale that a majority of the fragmented species are generated by premature transcriptional stops or mRNA hydrolysis. As such, the fragmented species predominantly do not contain both 5'-cap and poly(A) tail elements needed for protein expression.

The requested additional characterization data are provided below, along with new data that conclusively demonstrate that the fragmented mRNA species do not pose a risk for expression of truncated or other proteins.

Additional characterization of species isolated using ion pairing (IP) RP-HPLC

DS batch R427-P020.2-DS (Process 1) and batch 20Y513C501 (Process 2) samples were fractionated using ion pairing RP-HPLC to further characterize the intact and fragment mRNA species. The samples of

the active substance are representative of the finished product, as the fragment species profile is consistent between DS and DP, with only minor increases to the fragment content during DP manufacturing.

Figure 1 shows the ion pairing RP-HPLC chromatograms for batches R427-P020.2-DS and 20Y513C501. For both batches, two peaks are observed by IP-RP-HPLC. Fragment Analyzer electropherograms (**Figure 2**) demonstrate that Peak 1 consists almost entirely of fragmented species, consistent with the data provided previously in Assessment of Responses to CHMP Q01 – Quality 11-Dec-2020 (seq 0006). The Peak 2 electropherogram demonstrates that the predominant species is consistent with the main peak observed by fragment analyzer, with lower levels of fragments compared with the starting material.

Figure 1. Ion Pairing RP-HPLC Chromatograms for DS batches R427-P020.2-DS and 20Y513C501

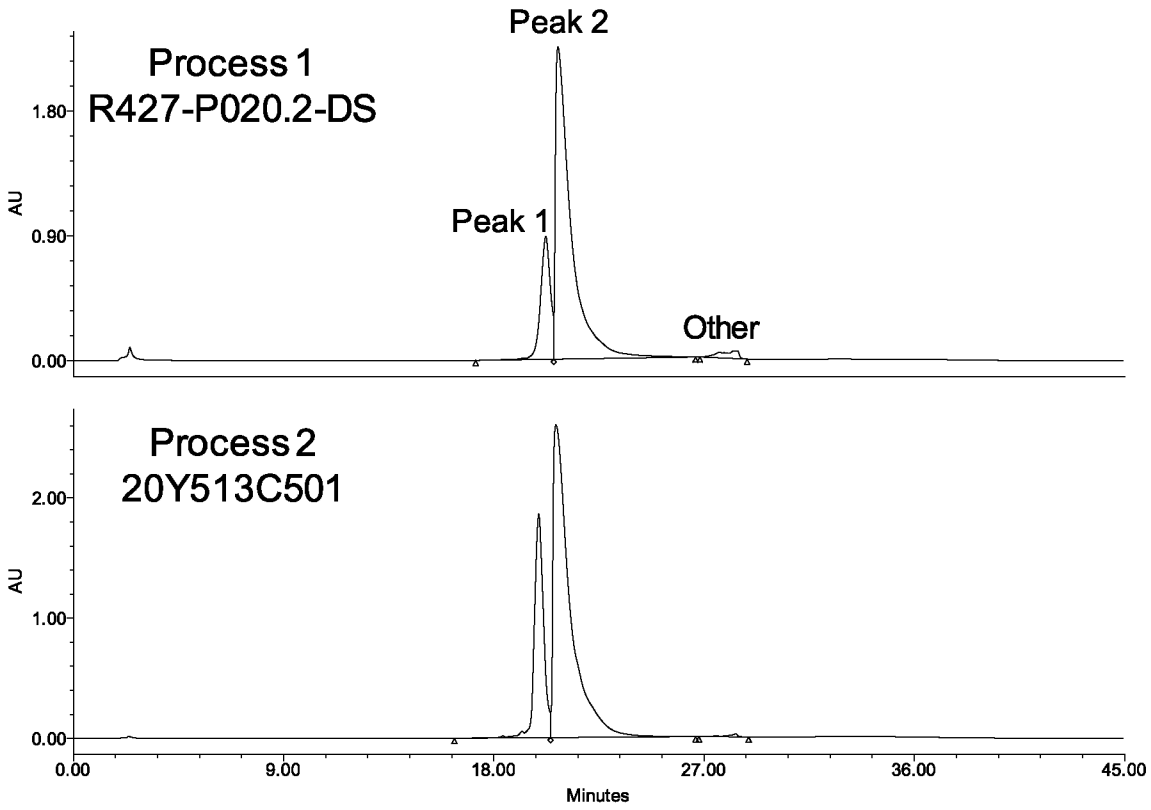
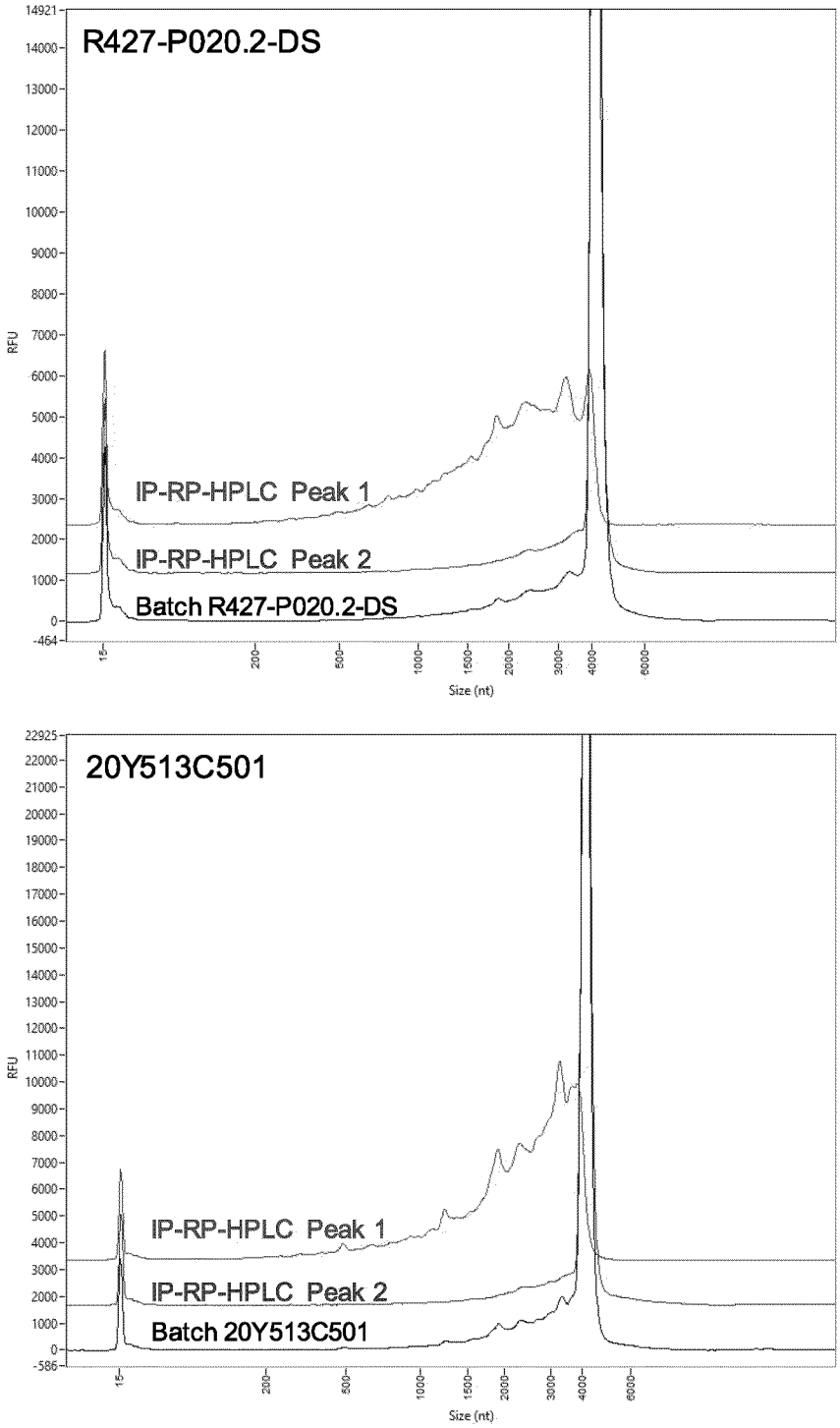


Figure 2. Fragment Analyzer Electropherograms of Peak 1, Peak 2, and Unfractionated DS

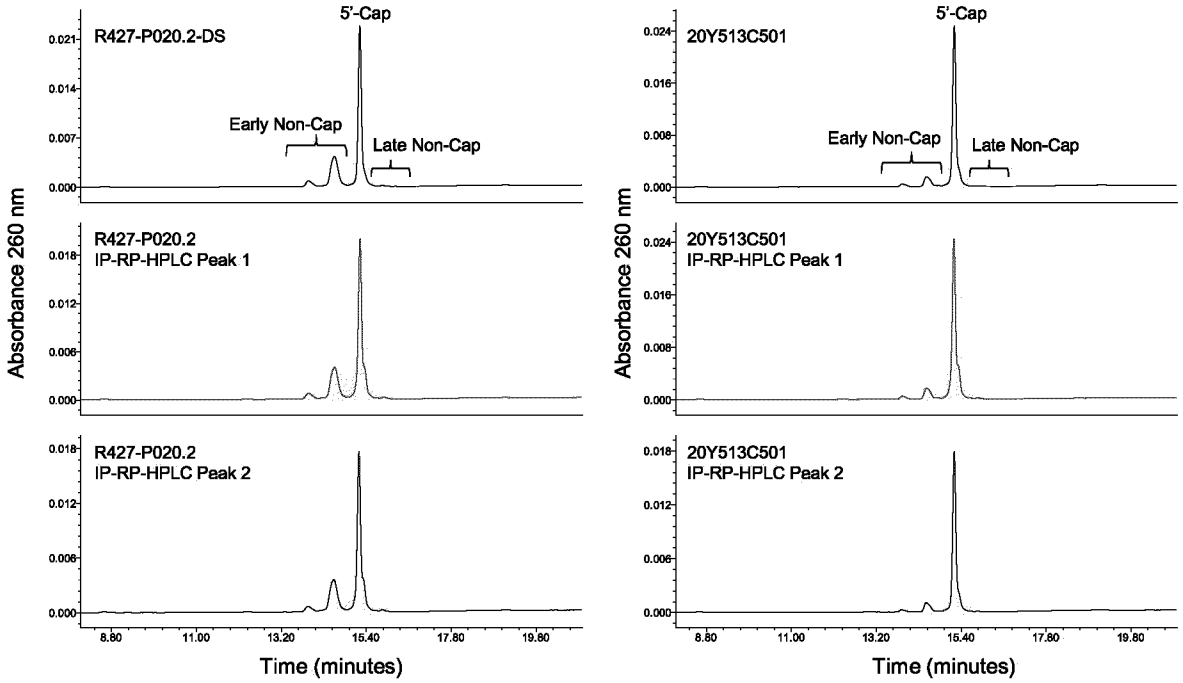


Peaks 1 and 2 from each DS batch (R427-P020.2-DS and 20Y513C501) were then isolated and characterized to compare 5'-cap and poly(A) tail length to that of unfractionated material. **Error! Reference source not found.** shows that 5'-cap and non-cap species content is unchanged across Peak 1, Peak 2, and unfractionated starting materials, consistent with previous characterization data. Additionally, comparable 5'-cap chromatographic profiles were observed for all six samples (Figure 3).

Table 1. 5'-Cap Species Content

Sample	Early Non-Cap (%)	5'-Cap (%)	Late Non-Cap (%)
R427-P020.2-DS	[redacted]	[redacted]	[redacted]
Peak 1 (R427-P020.2-DS)			
Peak 2 (R427-P020.2-DS)			
20Y513C501			
Peak 1 (20Y513C501)			
Peak 2 (20Y513C501)			

Figure 3. 5'-Cap Chromatograms of BNT162b2 DS, Peak 1, and Peak 2 Samples



In contrast to 5'-cap, characterization of the poly(A) tail length and distribution by IP-RP-HPLC demonstrates that the fragment species in Peak 1 lack poly(A) tail, while the Peak 2 and unfractionated starting DS samples show the expected length and distribution for the BNT162b2 poly(A) tail (**Figure 4**). These results support the conclusion that a significant proportion of fragment species in BNT162b2 are 5'-capped at levels consistent with the intact transcript, but predominantly lack the poly(A) tail, likely arising from premature transcriptional stops during production.

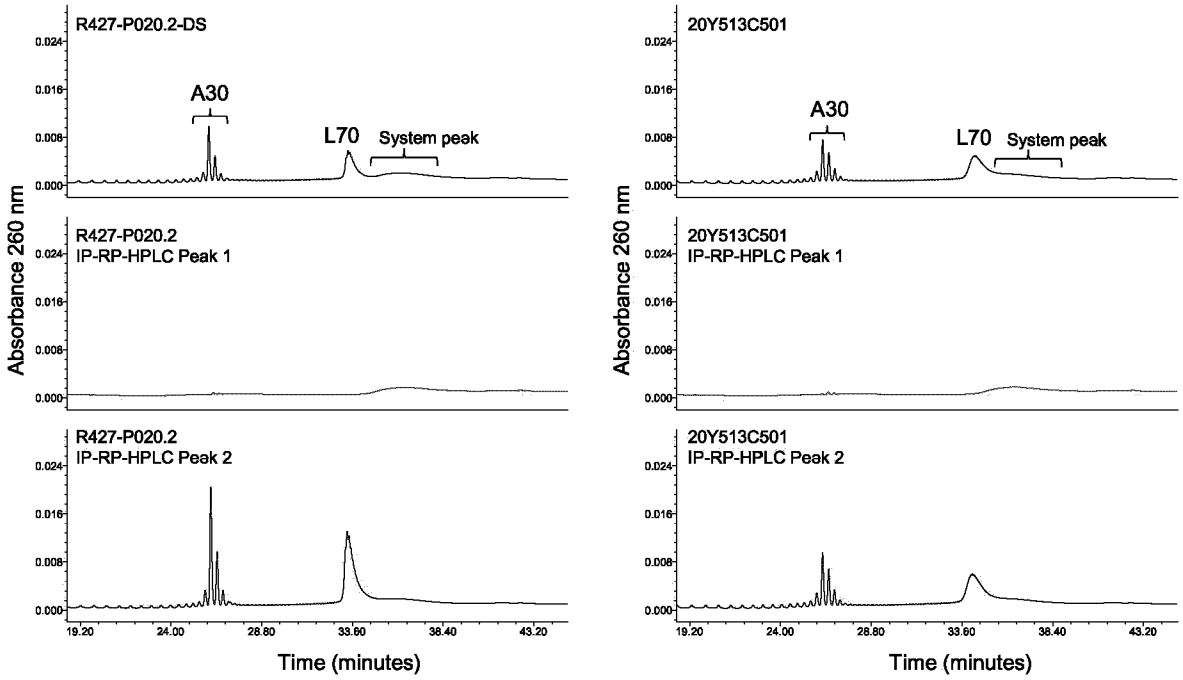
Quantitation of the poly(A) tail content by ddPCR further confirms that the fragment species in Peak 1 lack significant poly(A) tail content (< LOQ), while the Peak 2 and starting DS materials for both R427-P020.2-DS and 20Y513C501 contain [redacted] poly(A) tail content (**Error! Reference source not found.**). Additionally, the poly(A) tail content in Peak 2 fractions is consistently higher than the starting material, indicating enrichment of intact (i.e. poly-adenylated) transcripts in these samples.

Table 2. Poly(A) Tail Content

Sample	Poly(A) Tail Content (%)
R427-P020.2-DS	[redacted]
Peak 1 (R427-P020.2-DS)	
Peak 2 (R427-P020.2-DS)	
20Y513C501	
Peak 1 (20Y513C501)	
Peak 2 (20Y513C501)	

LOQ = Limit of Quantitation

Figure 4. Poly(A) Length and Distribution of BNT162b2 DS, Peak 1, and Peak 2 Samples



Assessment of the potential for translation into truncated S1S2 or other off-target proteins/peptides

To assess the potential for translation of mRNA fragments into truncated S1S2 proteins/peptides or other proteins/peptides, two orthogonal protein expression systems were utilized and translated proteins were evaluated by Western blot.

As shown previously in Section 3.2.S.2.6 Development History and Comparability Assessment, Western blot of BNT162b2-transfected cell lysates demonstrates expression of the S1S2 protein antigen. In this previous study, truncated protein species were not observed by Western blot in DS batches that contained up to 40% fragment species.

To test the hypothesis that transcripts require both 5′-cap and poly(A) to support protein translation, full-length transcripts lacking either 5′-cap or poly(A) tail were transfected into HEK-293 cells, and the resulting cell lysates were analyzed by Western blot using detection antibodies specific for the S1 or S2 domain. In contrast to BNT162b2 DS, which shows the expected banding pattern for the S1S2 protein antigen, S1S2 protein bands were not detected for the transcripts lacking either the 5′-cap (**Error! Reference source not found.**) or the poly(A) tail (**Error! Reference source not found.**). This control

experiment using full-length transcripts demonstrates that both 5'-cap and poly(A) tail are required for protein expression.

Figure 5. Full-Length Transcripts Require 5'-Cap for Translation

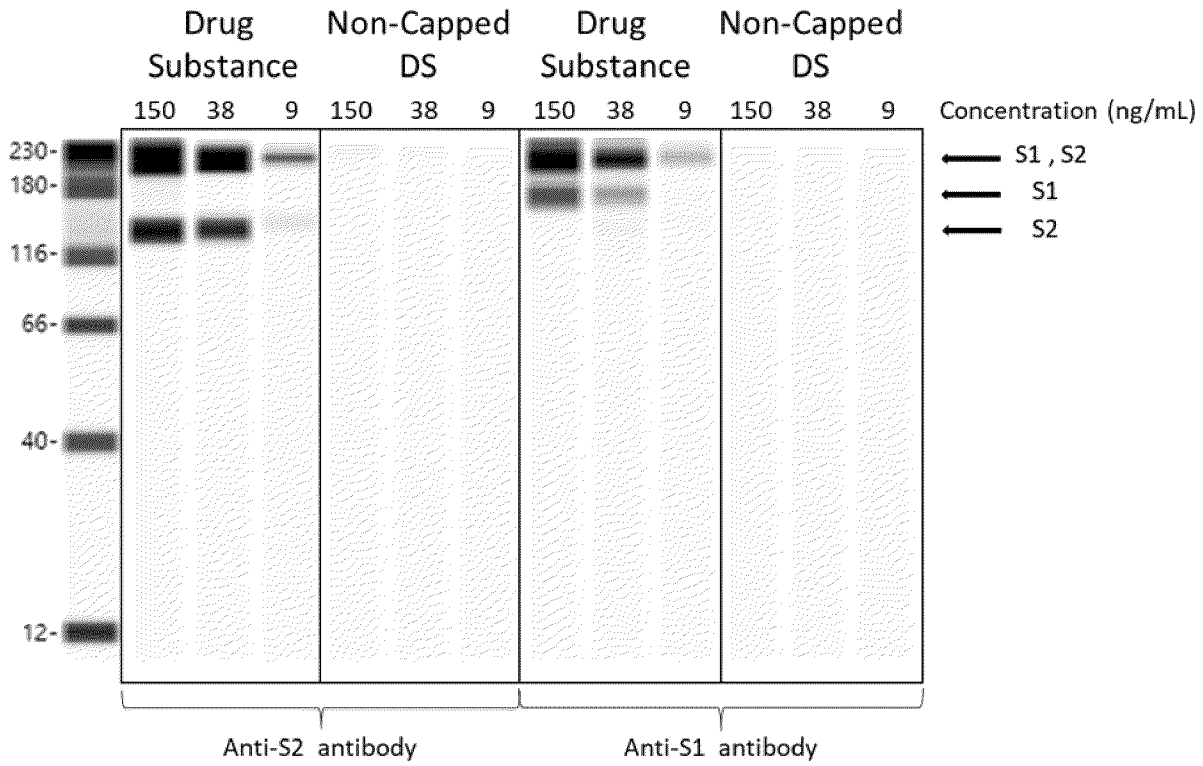
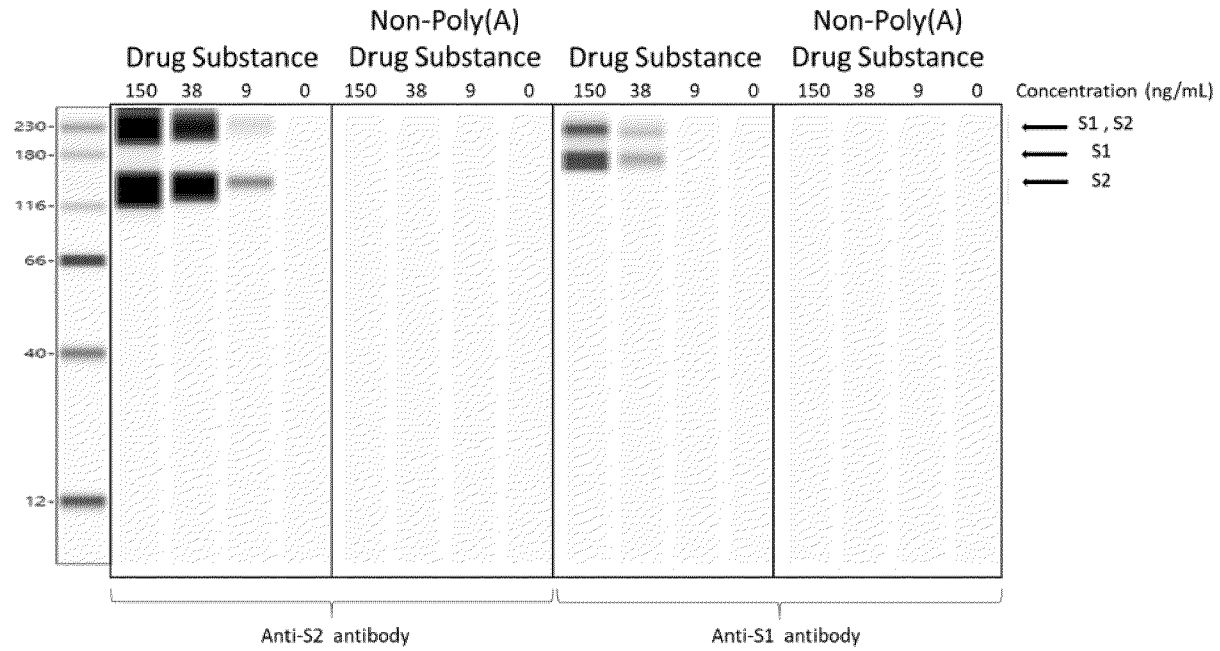


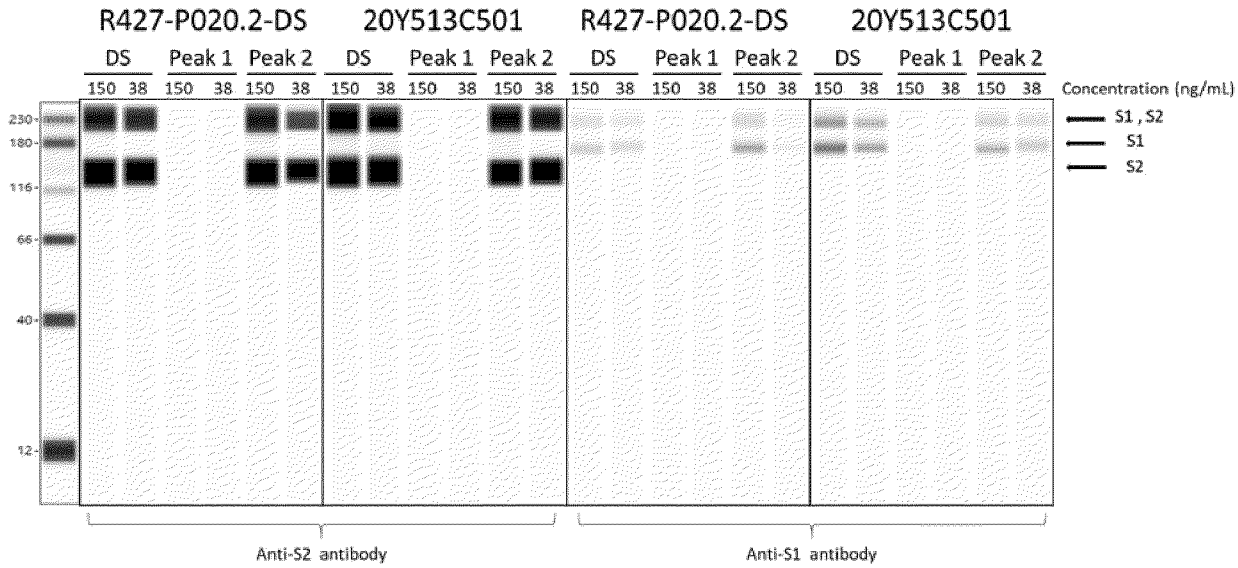
Figure 6. Full-Length Transcripts Require Poly(A) Tail for Expression



Consistent with the results using full-length mRNA transcripts, cells transfected with the starting DS batches (R427-P020.2-DS and 20Y513C501) or with Peak 2 material (isolated using IP-RP-HPLC above)

show comparable spike protein expression by Western blot, whereas cells transfected with the Peak 1 material comprised of fragmented species do not show evidence of protein expression (Figure 7). The results are consistent for both Process 1 and Process 2 batches, and both show a dose-dependent expression response for the unfractionated DS and Peak 2 samples.

Figure 7. Western Blot of Peak 1, Peak 2, and Starting DS Material

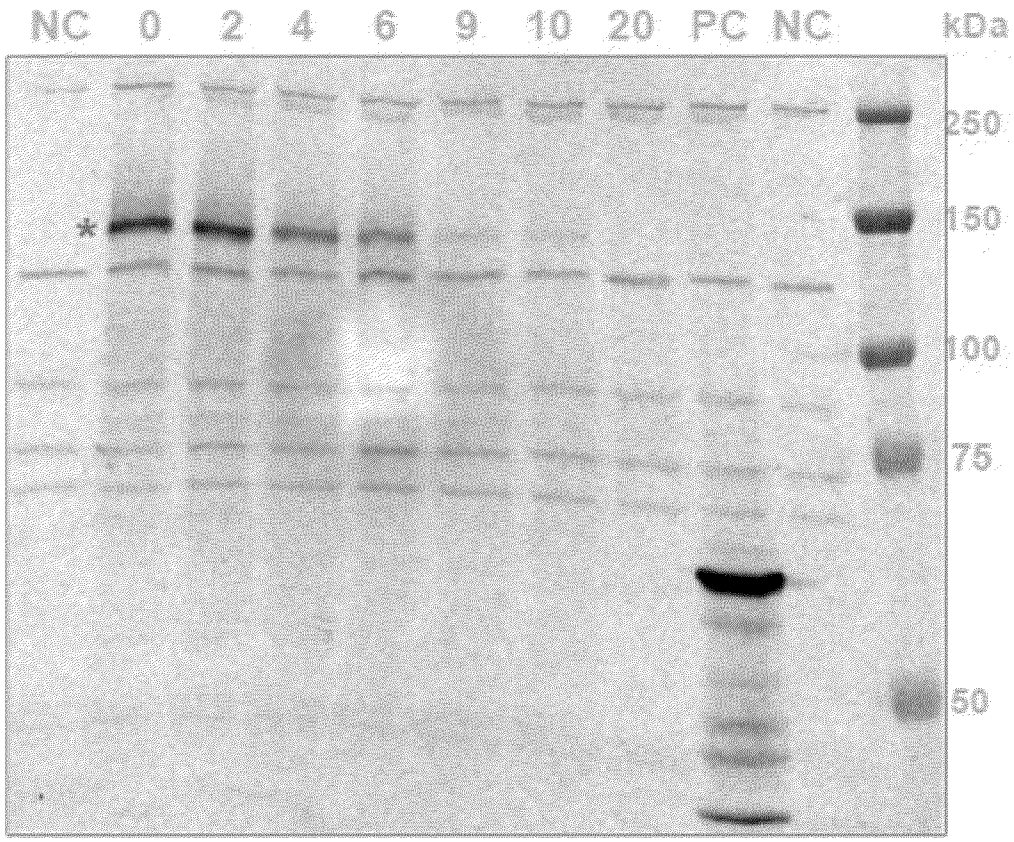


In a separate experiment, the potential for truncated transcripts to produce protein/peptides was evaluating using a cell-free in vitro expression system. BNT162b2 Process 2 batch 1071509 was intentionally degraded by exposure to elevated temperature, generating samples with various levels of fragmented species (Table 3). Fragment species in thermally degraded samples are generated by hydrolysis, which is the expected degradation pathway subsequent to in-vitro transcription (i.e. in the DP manufacturing process). The starting and degraded materials were subjected to in vitro expression using rabbit reticulocyte lysate, wherein biotinylated lysine is incorporated into newly translated proteins and peptides for detection by Western blot. This approach enables detection of both truncated S1S2 or other proteins/peptides, if present, without the need for protein/peptide-specific detection antibodies. After in vitro expression, the non-degraded BNT162b2 sample produced a protein approximately 140 kDa, which is consistent with the expected size of the aglycosylated S1S2 protein (denoted with a "*" in Figure 8 and additionally discussed in the response to part (c) below). For degraded samples, RNA integrity correlated with Western blot band intensity for the full-length protein; however, no truncated or other protein species were detected beyond the background bands observed in the negative control sample (NC).

Table 3. Thermally Degraded BNT162b2 Drug Substance

Sample	Degradation Time (min)	RNA Integrity (%)
1071509	0	[redacted]
1071509-2	2	
1071509-4	4	
1071509-6	6	
1071509-9	9	
1071509-10	10	
1071509-20	20	

Figure 8. In vitro translation of degraded BNT162b2 by Western blot



The Applicant states that additional characterization data presented here and provided previously support the following conclusions:

- Fragment species observed by Fragment Analyzer are consistent across DS Process 1 and Proc 2
  - Fragment species isolated using IP-RP-HPLC (Peak 1) contain 5'-cap, but lack poly(A) tail
  - IP-RP-HPLC Peak 2, which is predominantly comprised of intact RNA, include 5'-capped and poly-adenylated species
- 5'-cap and poly(A) tail are required for translation of the full-length BNT162b2 transcript
- Evaluation of full-length and degraded/fragmented transcript expression in two orthogonal expression systems shows no evidence of truncated S1S2 or other proteins or peptides

Response to c

The theoretical sizes of full-length and truncated S1S2 protein constructs (initially provided in Responses Q004– Quality 07-Dec-2020 (seq 0004)) are shown in Table4. As described above, the approximately 140 kDa protein expressed after in vitro expression of BNT162b2 is fully consistent with the theoretical size of the aglycosylated S1S2 protein (Figure 8). In this cell-free expression system, which lacks the cellular components required for glycosylation, the BNT162b2 expression indicates that a single, full-length or mature S1S2 protein is produced.

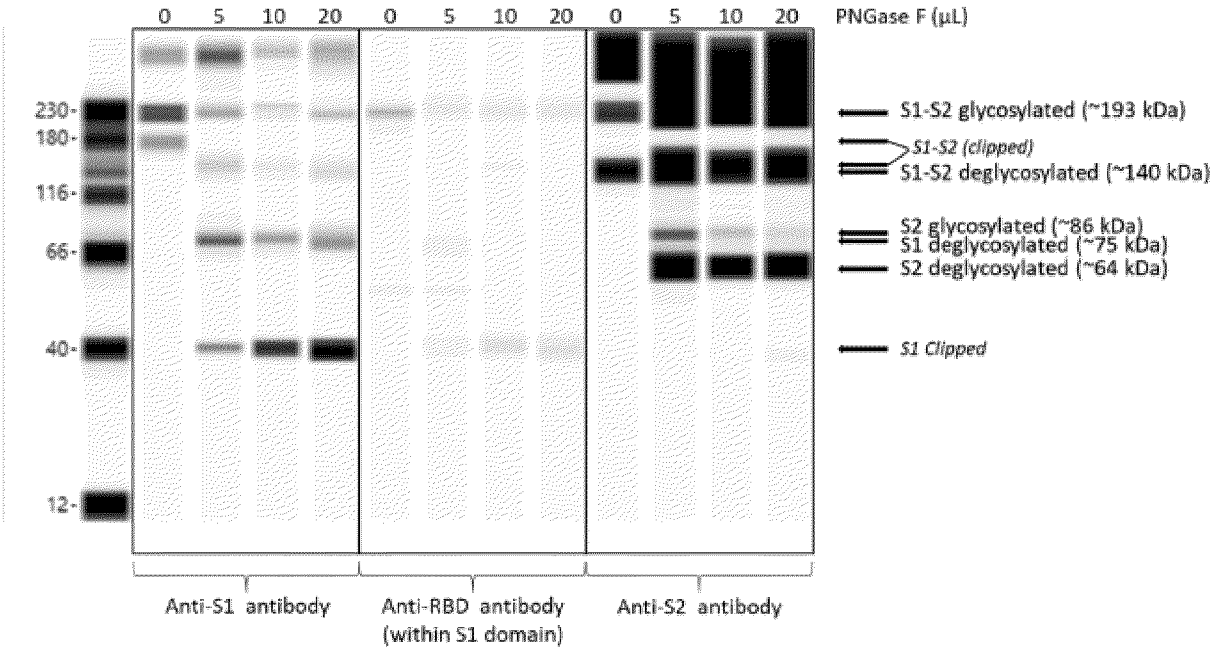
Table 4. Theoretical protein size after BNT162b2 expression

	S Protein with Signal Peptide	Mature S Protein	S1 Subdomain	S2 Subdomain
Residues	1-1273	13-1273	13-685	686-1273
# Amino Acids	1273	1261	673	588
Aglycosylated MW [Da]	141115.0	139755.3	75310.7	64462.6
Glycosylated MW[a] [Da]	192862.6	191502.9	105888.8	85632.0

Theoretical masses assume full occupancy of all 22 N-linked glycosylation sites with sialylated G2F+2NeuAc.

Additionally, the recommended enzymatic deglycosylation of the expressed proteins following BNT162b2 transfection into HEK-293 was pursued. In this study, increasing volumes of the de-glycosylating enzyme PNGase F were added to transfected cell lysates, and the resulting samples were analyzed by Western blot, with detection using antibodies specific for the S1 domain, the receptor binding domain (RBD, located within the S1domain), and the S2 domain. The resulting Western blot is shown in Figure 9. Upon PNGase F digestion, new bands ranging from ~40 kDa to ~140 kDa are detected. The ~140 kDa protein detected by the anti-Spike protein S1 domain antibody, anti-spike protein S2 domain antibody and anti-spike protein S1 domain RBD antibody is consistent with the deglycosylated full-length spike protein (S1S2). The band of ~75 kDa detected by anti-Spike protein S1 domain antibody and anti-spike protein S1 domain RBD antibody is consistent with deglycosylated S1 domain. The band of ~64 kDa detected only by anti-Spike protein S2 domain antibody is consistent with deglycosylated S2 domain. The new ~40kDa band detected by the anti-S1 and anti-RBD antibodies and the new band of ~90 kDa detected by anti-S2 antibody are believed to be the result of further proteolysis of the S1S2 spike protein within the cell lysate during PNGase F treatment steps. High molecular weight bands in the Western blot (i.e. > 230 kDa) likely result from aggregated spike proteins that are not fully denatured during sample preparation.

Figure 9. Deglycosylated proteins after BNT162b2 transfection are consistent with the S1S2 protein



Applicant's Overall Conclusions for the Fulfilment of Specific Obligation 1a, b, and c

The additional characterization data presented here fulfil the Specific Obligation 1 commitment and demonstrate that fragment species observed in BNT162b2 are not expressed as truncated or other

proteins/peptides. Further, additional characterization of the expressed protein using two orthogonal cell expression systems demonstrates that the expressed protein is the expected, full-length ~140kDa S1S2 protein. The data indicate that the S1S2 protein expressed in HEK-293 cells is post-translationally glycosylated, as confirmed by the observed removal of these glycans after addition of PNGase F to the cell lysate. Lastly, expression studies in rabbit reticulocyte lysate and in transfected cells show no evidence of truncated or other proteins expressed, including in samples degraded by elevated temperature to promote RNA fragmentation or in fragment species isolated using ion pairing RP-HPLC.

Assessor's comments:

SO1a+b

The Applicant has provided additional characterisation data on DS batch R427-P020.2-DS (Process 1) and batch 20Y513C501 (Process 2). Samples from the two batches were separated by ion pairing RP-HPLC and fractions were collected for characterisation. The fractions were analysed by fragment analyzer electrophoresis and it was demonstrated that IP-RP-HPLC peak 1 consists of the fragmented species, while peak 2 is consistent with the main peak observed by fragment analyzer electrophoresis.

Characterisation of the 5'-cap and poly(A) tail, both qualitatively and quantitatively, demonstrates that the fragmented species (IP-RP-HPLC peak 1) in general contain the 5'-cap but lack the poly(A)-tail. While the 5'-cap levels are similar for unfractionated DS, IP-RP-HPLC peak 1 and IP-RP-HPLC peak 2, the levels of poly(A) tail in peak 1 are below LOQ for both samples, but slightly higher for peak 2 as compared to unfractionated DS.

To assess the potential for translation of mRNA fragments into truncated S1S2 proteins/peptides or other proteins/peptides, two orthogonal protein expression systems were utilized and translated proteins were evaluated by Western blot. To test the hypothesis that transcripts require both 5'-cap and poly(A) to support protein translation, full-length transcripts lacking either 5'-cap or poly(A) tail were transfected into HEK-293 cells, and the resulting cell lysates were analysed by Western blot using detection antibodies specific for the S1 or S2 domain. The WB indicates that transcripts require both 5'-cap and poly(A) to accomplish protein translation, since no protein bands can be observed for the truncated species. However, WB is an epitope specific method, and truncated variants of the proteins could potentially be generated. Nevertheless, in line with the results obtained on full-length transcripts, cells transfected with the starting DS batches (R427-P020.2-DS and 20Y513C501) or with Peak 2 material (isolated using IP-RP-HPLC above) show comparable spike protein expression by Western blot, whereas cells transfected with the Peak 1 material, comprised of fragmented species, do not show evidence of protein expression.

In conclusion, it is sufficiently demonstrated that the major proportion of fragmented species from one representative Process 1 and one representative Process 2 DS batch contains the 5'-cap but lacks the poly(A) tail. The main peak was confirmed to represent the intact mRNA, containing both the 5'-cap and the poly(A) tail at levels comparable to the unfractionated bulk (5'-cap). Regarding the poly(A)-tail, the levels were found to be higher for the main peak as compared to unfractionated bulk, consistent with the finding that the fragmented species lack the poly(A) tail. **SO2b is considered solved.**

The potential for truncated transcripts to produce protein/peptides was further investigated using a cell-free in vitro expression system. For this purpose, one BNT162b2 Process 2 batch (1071509) was intentionally degraded by exposure to elevated temperature, generating samples with various levels of fragmented species. The starting and degraded materials were subjected to in vitro expression using rabbit reticulocyte lysate.

The inclusion of a cell-free in vitro translation system in the characterization of BNT162b2 DS is endorsed. Data provided demonstrate that the non-degraded BNT162b2 sample produced a protein approximately 140 kDa, consistent with the expected size of the aglycosylated S1S2 protein while for degraded samples, RNA integrity correlated with Western blot band intensity for the full-length protein. No truncated or other protein species were detected beyond the background bands observed in the negative control sample. However, no details are provided regarding the experimental setting for this characterization exercise which impairs the evaluation of data provided. The method used should be appropriately described, including conditions for in vitro reaction, antibodies used and choice of investigated material. Also, additional BNT162b2 batches with different levels of RNA integrity at release are expected to be included in the characterization exercise.

- Information should be provided regarding the settings of the cell-free in vitro translation study. The method used should be appropriately described, including conditions for in vitro reaction, antibodies used, the nature of the positive and negative controls and choice of investigated material. Release data for the batch used in the study should be provided and additional BNT162b2 batches with different levels of RNA integrity at release, ideally having historically low and high levels of RNA integrity, should be included in the characterization exercise.

SO1c

A table is provided presenting the theoretical masses of the S-protein and the subdomains in glycosylated and non-glycosylated forms. It should be noted that there are 22 N-glycosylation sites and that, a theroretical mass is only calculated for one single glycoform at all sites. The molecular weight obtained for the protein band in the cell-free expression system is consistent with the calculated mass of the deglycosylated protein.

Deglycosylation of the expressed proteins following BNT162b2 transfection into HEK-293 was performed. WB results obtained by three different antibodies, specific for the S1 domain, the receptor binding domain and the S2 domain, respectively, are presented. Overall, the quality obtained for WB by the anti-S2-antibody is considered poor, possibly due to poor optimisation of the methods. However, the results obtained with the anti-S1 antibody demonstrates that a protein of size 140 kDa is obtained upon PNGase digestion. Bands corresponding to the S1 and S2 subdomains are identified after PNGase digestion. These findings are not explained nor discussed by the Applicant, and such a discussion would have been highly appreciated. Nevertheless, the Applicant has confirmed the identities of the WB bands obtained by the in vitro expression assay. **SO1c is considered solved.**

- The updated characterisation data included in the response document should be included in Module 3.2.S.2.6 and/or 3.2.S.3 of the dossier.

Conclusion

Issue partly solved. Additional information regarding SO1a remains to be provided. SO1b and c are considered solved.

6.1.2. Control strategy - SO2

In order to ensure consistent product quality, the MAH should provide additional information to enhance the control strategy, including the active substance and finished product specifications.

a) The active substance and finished product specifications acceptance limits, being wider than the actual ranges for which clinical experience is available now, should be re-assessed and revised as appropriate, as further data becomes available from ongoing clinical trials and in line with

manufacturing process capability and stability data of the product. Comprehensive data should be provided comprising batch analyses of a suitable number of commercial batches as well as analyses of batches that have been used in the (ongoing) clinical trials.

b) Poly(A) tail length is considered a critical attribute, which should be controlled on each batch, even though comparable results were obtained until now. An active substance specification to control poly(A) length should be introduced. A suitable method should be developed and appropriate acceptance criteria should be set.

c) The poly(A) tail percentage is considered a critical attribute, but uncertainties remain on the suitability of the method. Additional data should be provided to support the suitability of the method used for %poly(A) tail or an alternative suitable assay should be developed and introduced. The Applicant should evaluate any potential overestimation of poly(A) tail by the ddPCR method. The %poly(A) tail should be characterised following any future active substance process changes.

d) Since mRNA integrity and polydispersity are CQAs for the efficacy of the medicinal product, the finished product acceptance criteria for these parameters should be revised as further data becomes available from ongoing clinical trials and in line with manufacturing process capability.

e) Additional data should be provided to support the suitability of the method used for potency determination or an alternative suitable assay for this purpose should be developed and introduced. Then the finished product acceptance criteria for potency should be revised accordingly.

f) Lipid-related impurities as well as potential presence of lipid-RNA adducts in the finished product should be further evaluated. In particular, late migrating species (LMS) observed in the capillary gel electrophoresis (CGE) for some finished product batches should be included in the evaluation. The LMS may impact the QTPP of the medicinal product and therefore an appropriate control strategy for the LMS should be introduced, suitably justified and provided for assessment during Q2 2021. See separate assessment report for EMEA/H/C/005735/II/0054/G.

Response SO2a

The active substance and finished product specification acceptance criteria have been reassessed and revised, as appropriate, following the manufacture of additional batches and lots, respectively. At the time of the MAA closing sequence (0006) submission, data from 10 representative (process 2) active substance (DS) batches and from 6 commercial / emergency supply finished product (DP) lots were considered in combination with DS batches and DP lots manufactured for nonclinical and clinical use. The updated data sets include 42 commercial / emergency supply DS batches and 83 commercial / emergency supply DP lots, respectively. All DS batch and DP lot release data available through mid-February 2021, including process performance qualification (PPQ) batches and lots for both initial DS manufacturing nodes (ACMF, Pfizer and Mainz, BioNTech/Rentschler) and the initial DP fill/finish manufacturing nodes (Puurs, Pfizer and Kalamazoo, Pfizer).

These data have been assessed statistically, where appropriate, and acceptance criteria have been re-evaluated considering clinical and real-world experience, manufacturing process capability and stability data.

The revisions made to the proposed specifications for BNT162b2 DS and DP, as well as references to further descriptions and justifications. Updates to the DS and DP specifications and the associated justifications are provided in Section 3.2.S.4.1 Specification, Section 3.2.S.4.5 Justification of Specification, Section 3.2.P.5.1 Specification(s) and Section 3.2.P.5.6 Justification of Specification(s).

Response SO2b

A new method to evaluate poly(A) tail length has been developed and validated for release testing of the active substance (Section 3.2.S.4.2 IP-RP-HPLC and Section 3.2.S.4.3 IP-RP-HPLC). A specification acceptance criterion has been established to confirm poly(A) tail length for each DS batch on release (Section 3.2.S.4.1 Specification and Section 3.2.S.4.5 Justification of Specification).

Response SO2c

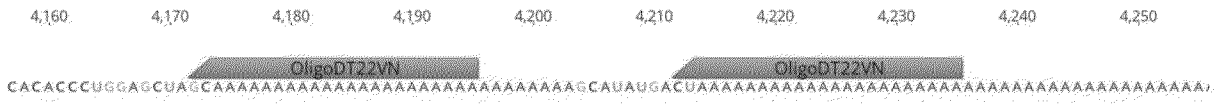
Poly(A) tail content determination by the ddPCR method is a two-step process, involving reverse transcription of mRNA into cDNA followed by digital droplet quantitation of the cDNA. The method qualification exercise demonstrated suitability for poly(A) content determination, and subsequent method validation confirms the method suitability (discussed in greater detail below). Historical test results (e.g. poly(A) content > 100%) have indicated a potential overestimation of poly(A) tail content; however, the method precision and measurements support its ability to differentiate samples by their poly(A) content. In addition, the method can definitively confirm when a sample lacks poly(A) tail, as demonstrated in Module 1 Specific Obligation 1 and in linearity spiking studies below. A method assessment was undertaken to identify potential causes of poly(A) content overestimation, and the method suitability was re-evaluated in the context of these potential causes of overestimation.

Assessment of potential overestimation of poly(A) tail by the ddPCR method

Over-Priming of OligoDT22VN

In the first step of the ddPCR method, conversion of mRNA into cDNA, a reverse transcription primer anneals to the poly-adenylation sequence of the BNT162b2 mRNA. Over-priming of the reverse transcription primer is a potential mechanism that would result in overestimation of the final poly(A) content. Because BNT162b2 contains two poly(A) segments, containing 30 and 70 adenosines separated by a 10 nucleotide linker, the reverse transcription primer (OligoDT22VN) can potentially anneal to either or both of these segments as shown in **Error! Reference source not found.**. In the event the reverse transcription primer anneals to both sites, two cDNA copies can be generated. Because poly(A) content is calculated from the measured concentration in the second step of the method (ddPCR quantitation), compared to the theoretical input, the generation of two cDNA's from a single mRNA strand can result in an over-estimation of the poly(A) content (e.g. above 100%).

Figure 1. OligoDT22VN Reverse Transcription Primer Annealing Sites



Fragment Bias

A second potential cause of poly(A) tail content over-estimation is the presence of fragments derived from terminated transcripts. The poly(A) content is calculated by dividing the measured poly(A) tail copies/µL (from the ddPCR assay) by the input number of mRNA copies (targeting [redacted] copies/µL; see Equation 1). The target concentration corresponding to [redacted] copies/µL is derived from the theoretical molecular weight (MW) of the mRNA transcript (Equation 2). Prematurely terminated transcripts in the sample; however, will decrease the average molecular weight for a given sample due to the smaller MW of the fragment species. As a result, the sample can contain more than [redacted] mRNA copies/µL at the target concentration (i.e. the denominator constant of [redacted] copies/µL in Equation 1 would be slightly lower than the true copy number), which can lead to slight overestimation of the poly(A) tail content in Equation 1 below. It should be noted that any fragments formed due to full-length mRNA chain breakages (i.e. hydrolysis), occurring outside of the ddPCR poly(A) target region, will not affect the

measurement of poly(A) content because this region would still be available as a template for ddPCR analysis. Nevertheless, the fragment species content is controlled through specification acceptance criteria for RNA integrity, and characterization studies (see Module 1 Specific Obligation 1) demonstrate consistency in DS fragment species profiles.

Equation 1 – poly(A) tail content determination:

$$\text{Poly(A) Tail (\%)} = \frac{\text{mean } 3' \left(\frac{\text{copies}}{\mu\text{L}}\right)}{1000 \left(\frac{\text{copies}}{\mu\text{L}}\right)} \times 100$$

Equation 2 – mRNA concentration determination for 1000 copies/µL:

$$\text{Target concentration} \left(\frac{\text{fg}}{\mu\text{L}}\right) = 1000 \frac{\text{copies}}{\mu\text{L}} \times \frac{1 \text{ mol}}{6.022 \times 10^{23} \text{ copies}} \times \mathbf{MW} \left(\frac{\mathbf{g}}{\mathbf{mol}}\right) \times \frac{10^{15} \text{ fg}}{1 \text{ g}}$$

Re-EvaluF96ation of Method Suitability

Potential for Over-Priming and Fragments to Impact Method Consistency

To determine if over-priming of the reverse transcription primer affects method consistency, data collected from internal control sample (20Y513C201-RM) were evaluated (**Error! Reference source not found.**). Over 104 instances, the average poly(A) content by ddPCR was observed to be 96% with a 9.6% CV (among 10 Analysts across 2 sites), which demonstrates the consistent performance of the method, regardless of over-priming.

The potential for fragment species to impact the ddPCR method is considered low, due to process controls and the RNA integrity specification, which ensure consistent fragment levels in each drug substance batch.

Table 5. Batch 20Y513C201-RM Internal Control Trending

# Instances	Average Poly(A) Tail (%)	Standard Deviation	CV (%)
[redacted]			

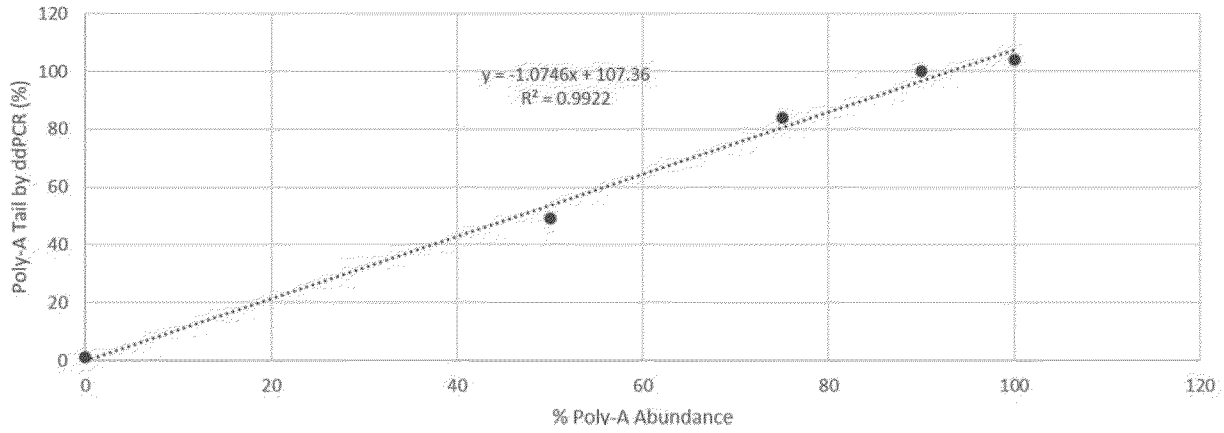
While fragment species can contribute to overestimating poly(A) tail content, the presence of fragments is not expected to impact method consistency. Fragment species content has remained consistent throughout the manufacturing history, and the attribute is controlled through specification acceptance criteria for RNA integrity.

ddPCR is Sensitive to Changes in Poly(A) Tail Content

The Applicant has conducted further evaluation to demonstrate the capability of the ddPCR method to detect changes in poly(A) tail content. Samples with varying levels of poly(A) tail content (0%, 50%, 75%, 90% and 100%) were prepared by combining RNA drug substance completely lacking a poly(A) tail (0%) and a representative commercial RNA drug substance (established as 100% poly(A) tail for this experiment). The samples were normalized to 1000 copies per µL, as per the test method, and analyzed by ddPCR to quantitate % poly(A) tail content. The results of this study are presented in **Error!**

Reference source not found.. A linear response is observed (slope = 1.0746, R^2= 0.9922), which demonstrates the ddPCR method is capable of accurately differentiating poly(A) tail content across a wide range.

Figure 2. Linearity of Poly(A) Tail Content by ddPCR (0% to 100%)



Conclusions by MAH

The current method assessment identified potential sources for overestimation of poly(A) tail content by ddPCR. However, a subsequent evaluation has demonstrated that any potential over-priming is consistent and the impact of fragment species on the sample concentration estimate is consistent, based on the control of intact RNA content through the drug substance control strategy. Furthermore, the method has been shown to be sensitive to detecting mRNA samples lacking a poly(A) tail, further verifying the intended purpose of the method. Therefore, the Applicant intends to continue using the ddPCR method, which appropriately controls the poly(A) tail attribute in combination with the newly developed method for poly(A) tail length by IP-RP-HPLC in drug substance release testing.

Response SO2d

The sponsor has reassessed RNA integrity and polydispersity of the BNT162b2 product following additional manufacturing experience. A summary of the drug product data set used for this evaluation is described in the response to Specific Obligation 2a.

The acceptance criteria for RNA integrity (release criteria for drug substance and release and stability criteria for drug product) were recently tightened as part of the approved variation to facilitate point of use storage and administration (EMEA/H/C/005735/IB/031G), which partially fulfilled SO2d. Section 3.2.S.4.5 Justification of Specification and Section 3.2.P.5.6 Justification of Specification contain the statistical assessment and justification for setting the acceptance criteria for drug substance and drug product, respectively.

Similarly, the reassessment and justification for the acceptance criterion for the polydispersity quality attribute (PDI) is described in Section 3.2.P.5.6 Justification of Specification.

Response SO2e

Additional method optimization, evaluation of assay variability and replication strategy for in vitro expression (IVE) assay were performed. Data presented supported the suitability of the method used for potency determination.

Response SO2f

See separate assessment report for EMEA/H/C/005735/II/0054/G.

Assessor´s comments:

For assessment of the responses to SO2 a to e see the headings for the separate eCTD sections below.

6.1.3. Control of Drug Substance (3.2.S.4)

6.1.3.1. Specification (3.2.S.4.1)

Table 3.2.S.4.1-1 BNT162b2 Drug Substance Specification

Quality Attribute	Analytical Procedure	Procedure Number(s)	Acceptance Criteria
Composition and Strength			
Clarity	Appearance (Clarity)	Ph. Eur. 2.2.1	≤ 6 NTU
Coloration	Appearance (Coloration)	Ph. Eur. 2.2.2	Not more intensely colored than level 7 of the brown (B) color standard
pH	Potentiometry	Ph. Eur. 2.2.3, USP <791>	[redacted]
Content (RNA Concentration)	UV Spectroscopy	TM100010308 [b] RL-SOP-02645 [d] SOP-10071 [f] LAB-37658 [c]	[redacted] mg/mL
Identity			
Identity of Encoded RNA Sequence	RT-PCR[a]	TM100010407 [b] TM-072-038 [e] LAB-37698 [c]	Identity confirmed
Purity			
RNA Integrity	Capillary Gel Electrophoresis	TM100010392 [b] TM-072-039 [e] LAB-41057 [c]	[redacted] intact RNA
5'- Cap	RP-HPLC	TM100010578 [b] PAN-1239-K [g] PAN-1253-K [g] LAB-37879 [c]	
Poly(A) Tail	ddPCR	TM100010379 [b] PAN-1280-K [g] LAB-38823 [c]	
Poly(A) Tail Length	IP-RP-HPLC [a]	TM100010391 [b] SOP-501868 [f]	Poly(A) tail length confirmed
Process Related Impurities			
Residual DNA Template	qPCR [a]	TM100010388 [b] TM-072-028 [e] LAB-42528 [c]	[redacted] ng DNA/mg RNA
Product Related Impurities			
dsRNA	Immunoblot [a]	PAN-1356-K [g] TM100010474 [b] LAB-42529 [c]	[redacted] pg dsRNA/µg RNA

Quality Attribute	Analytical Procedure	Procedure Number(s)	Acceptance Criteria
Safety			
Bacterial Endotoxin	Endotoxin (LAL)	Ph. Eur 2.6.14, USP <85>, JP 4.01	≤ 12.5 EU/mL
Bioburden	Bioburden [a]	Ph. Eur. 2.6.12, USP <61>, JP 4.05	≤ 1 CFU/10 mL

a. Assay not performed on stability.
b. Analytical Procedure at Pfizer Analytical Research and Development
c. Analytical Procedure at Pfizer Global Supply, Andover, MA, USA
d. Analytical Procedure at Rentschler
e. Analytical Procedure at BioNTech, Mainz
f. Analytical Procedure at BioNTech, Marburg
g. Analytical Procedure at BioNTech IMFS

Abbreviations: NTU = Nephelometric Turbidity Units; B = brown; RT-PCR = reverse transcription polymerase chain reaction; RP-HPLC = reversed phase-high performance liquid chromatography; ddPCR = droplet digital PCR; IP-RP-HPLC = ion-pairing reversed phase-high performance liquid chromatography; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amebocyte lysate; EU = endotoxin unit; CFU = colony forming unit; NA = not available

Assessor´s comments:

The updated DS specification is provided as anew document in eCTD sequence 0144 and not as replaced document. This is not found acceptable; the new document should be provided as replaced document.

- The updated DS specification should be provided as a replaced document and not as a new document. Module 3.2.S.4.1 of the dossier should be updated accordingly.

6.1.3.2. Analytical procedures (3.2.S.4.2)

Ion pair-reverse phase-HPLC (IP-RP-HPLC)

The purpose of this analytical procedure is to measure the length of the 3' polyadenylic acid (Poly(A)) tail in BNT162b2 drug substance (DS) mRNA. DS test samples are digested using RNase T1 and RNase A followed by ion pair-reversed phase-high performance liquid chromatography (IP-RP-HPLC) with UV detection.

RNase T1 specifically cleaves single-stranded RNA on the 3'-end of guanosine (G) residues, and RNase A cleaves single-stranded RNA on the 3'-end of uridine (U) and cytidine (C) residues. The resulting products are an approximately 30 Poly (A) nucleotide tail (A30) and an approximately 70 Poly(A) nucleotide tail (L70) mixed with other shorter nucleotides. The 30 Poly(A) and the 70 Poly(A) nucleotide tails are separated by IP-RP-HPLC. The lengths of the Poly(A) tails are confirmed and reported using relative retention time (RRT) compared to a well characterized mRNA DS reference material (RM).

The IP-RP-HPLC operating parameters are provided in Table 3.2.S.4.2-4.

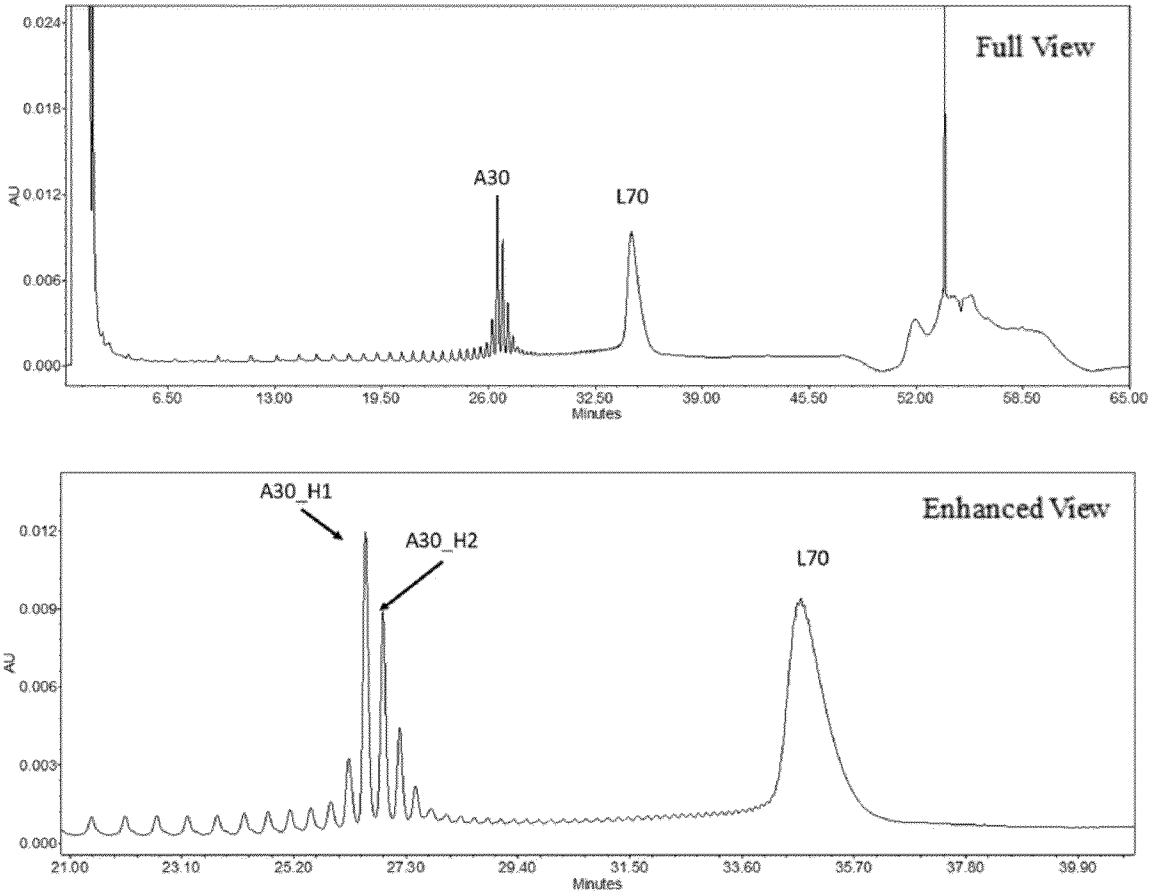
Table 3.2.S.4.2-4. Chromatographic Conditions and System Operating Parameters

Column	[redacted]	
Column temperature		
Autosampler temperature		
UV detection		
UV reference[a]		
Injection volume		
Flow rate		
Mobile phase A:	[redacted]	
Mobile phase B:		
Gradient		
Time (minutes)	**Mobile Phase A (%)**	**Mobile Phase B (%)**
0	[redacted]	[redacted]
2		
30		
50		
52		
57		
59		
65		

a. instrument dependent, may not be required.

Representative chromatograms of the blank, RM and DS are presented in the dossier, Figure 3.2.S.4.2-2 for the RM is included below.

Figure 3.2.S.4.2-2. Representative Chromatograms of RM



System suitability is assessed by analysis of the blank and RM. Assay acceptance is assessed by analysis of the RM, and sample acceptance is assessed by analysis of the TS. The criteria listed in the dossier and includes requirements for visually comparable chromatographic profiles, RSD of the retention time, resolution and tailing.

Provided the system suitability, assay, and sample acceptance criteria are met, and the RRT of the A30 and L70 peaks are [redacted], the TS is reported as "poly(A) tail length confirmed".

6.1.3.3. Validation of analytical procedures

The ion pair-reversed phase-high performance liquid chromatography (IP-RP-HPLC) analytical procedure for the determination of poly(A) tail length in BNT162b2 drug substance (DS) has been validated in conformance with ICH Q2(R1) guidelines. During the validation, the performance of the test method was evaluated against a set of defined acceptance criteria for specificity and robustness. A summary of the validation parameters, experimental design, acceptance criteria, and results is presented in Tables 3.2.S.4.3-1 and 3.2.S.4.3-1 for the sites Pfizer-ARD and PGS-AND and BioNTech Marburg respectively.

Table 3.2.S.4.3-1. Validation - Pfizer-ARD and PGS-AND

Validation Parameter	Experimental Design	Acceptance Criteria	Validation Results
Specificity	Positive identity and negative challenge samples were analyzed according to the analytical procedure at ARD-STL, ARD-AND, and PGS-AND with 4 instances, analysts and columns Positive validation sample: BNT162b2 DS Negative validation samples: Formulation buffer Challenge Sample 1[b] Challenge Sample 2[c] Challenge Co-mix Sample 1[d] Challenge Co-mix Sample 2[e]	A positive response must be observed for BNT162b2 DS A negative response must be observed for formulation buffer, challenge samples, and challenge co-mix samples	A positive response was observed for BNT162b2 DS A negative response was observed for formulation buffer, challenge samples, and challenge co-mix samples
Robustness[a] (In-vial stability)	Samples were prepared according to the analytical procedure, stored in the autosampler under test method conditions, and injected at T0, T12, T23, T34, T45, and T49 hr	N/A	Sample stability was demonstrated for up to 49 hr in the autosampler under test method conditions prior to analysis
Robustness[a] (Alternate column and instrument)	Samples were analyzed according to the analytical procedure using 2 different columns and instruments	Report result	Similar results were obtained using the different column and instrument combinations
Robustness[a] (Storage stability post digestion)	Samples were analyzed immediately after digestion and after 20 days stored at -80 °C	Report result	No appreciable change in the chromatograms was observed between T0 and T20 days at -80 °C

Abbreviations: nt = nucleotides, T = time

a. Robustness was evaluated during method qualification.

b. Challenge sample 1 contained a 25 Poly(A) length (25 nt) single stranded RNA oligonucleotide.

c. Challenge sample 2 contained a 40 Poly(A) (40 nt) length single stranded RNA oligonucleotide.

d. Challenge co-mix sample 1 contained a 1:1 co-mix of BNT162b2 DS and 25 Poly(A) length single stranded RNA oligonucleotide.

e. Challenge co-mix sample 2 contained a 1:1 co-mix of BNT162b2 DS and 40 Poly(A) length single stranded RNA oligonucleotide.

Table 3.2.S.4.3-2. Validation – BioNTech Marburg

Validation Parameter	Experimental Design	Acceptance Criteria	Validation Results
Specificity	Positive identity and negative challenge samples were analyzed according to the analytical procedure at IMFS Positive validation sample: BNT162b2 DS Negative validation samples: DS buffer Challenge Sample 25 Challenge Sample 40 Challenge Co-mix Sample 20 Challenge Co-mix Sample 40	A positive response must be observed for BNT162b2 DS A negative response must be observed for challenge samples, the co-mix-samples and the DS buffer sample	A positive response was observed for BNT162b2 DS A negative response was observed for challenge samples, the co-mix-samples and the DS buffer sample
Robustness (Alternate column)	Samples were analyzed according to the analytical procedure using 2 different columns	The chromatograms of the 2 columns have to be visually comparable	The two chromatograms were visually comparable
Robustness (Sample incubation time)	Three samples were prepared and incubated at 3, 3.5 and 4 hours	The relative difference of RRT of peaks of interest (A30[a] and L70[b]) with regard to standard time [redacted]	[redacted]
Robustness (In-vial stability)	Samples were prepared according to the analytical procedure, and injected at T7, T17, T27, T41, T51 and T64 hours after preparation	RSD of RRT of peaks of interest (A30[a] and L70[b]): [redacted]	Peak A30: RSD = [redacted] Peak L70: RSD =
Robustness (Storage of intermediate sample preparations)	Samples were analyzed immediately after digestion and after 4 days stored at ≤ -60 °C	The two chromatograms have to be visually comparable	The two chromatograms were visually comparable
Robustness (Additional freeze/thaw cycles of DS reference sample)	Reference standard was thawed, analyzed and frozen (-20 °C) again. After 7 days the standard was thawed and analyzed again.	The relative difference in RRT of peaks of interest (A30[a] and L70[b]) with regard to sample without freeze/thaw cycle differ [redacted]	RRT A30: Δ = [redacted] RRT L70: Δ =

a. Peaks used to confirm the nucleotide length prior to the linker region
b. Peaks used to confirm the nucleotide length post-linker
Abbreviations; DS = drug substance; RRT = relative retention time; RSD = relative standard deviation; Δ = difference

Assessor´s comments on S.4.2 and S.4.3:

Ion pair-reverse phase-HPLC (IP-RP-HPLC) has been introduced as a new drug substance release method to measure the length of the poly(A) tail to fulfill SO2b. The method description is found adequate and includes detail on operating parameters, representative chromatograms and system suitability criteria.

Summaries of the validation exercise performed for the new IP-RP-HPLC method are presented. These include short descriptions of the validation parameters, experimental design as well as validation results. The information is presented in two different tables, one table for the sites Pfizer Analytical Research and Development (ARD), Chesterfield, MO and Andover, MA (ARD-STL and ARD-AND), and Pfizer Global Supply, Andover, MA (PGS-AND) and one table for the BioNTech Marburg site.

In general, the information provided seems to be relevant in supporting the suitability of the analytical procedure for their intended use at each site. However, the level of details provided is considered too limited to allow for a proper assessment and more detailed summaries of the validation results should be provided.

Conclusion:

- The level of details provided for the validation exercise for the new IP-RP-HPLC is considered too limited to allow for a proper assessment. More detailed method validation summaries including test results for individual samples, calculations, chromatograms etc. should be provided to support equal and adequate performance of the test method at all test sites. Module 3.3.S.4.3 of the dossier should be updated accordingly.

6.1.3.4. Justification of Specifications (3.2.S.4.5)

Poly(A) Tail Length

Poly(A) Tail Length is a quality attribute corresponding to the length of the poly(A) tail as assessed by digestion using RNase A and RNase T1 followed by separation by IP-RP-HPLC to evaluate the resulting poly(A) segments, A30 and L70. The method for this attribute has been implemented recently, and the method assesses the length of these two segments relative to that of a reference material by assessment of relative retention time. Table 3.2.S.4.5-18 contains results for representative process 2 drug substance batches tested retrospectively by this method. The acceptance criterion for this method for assessment of drug substance at release is shown in Table 3.2.S.4.5-19.

Table 3.2.S.4.5-18. BNT162b2 Process 2 Drug Substance Retrospective Poly(A) Tail Length Testing Results

Batch	Result
20Y513C101	Poly A Tail Length Confirmed
20Y513C401	Poly A Tail Length Confirmed
20Y513C701	Poly A Tail Length Confirmed
20Y513C1001	Poly A Tail Length Confirmed
20Y513C1301	Poly A Tail Length Confirmed
20Y513C1601	Poly A Tail Length Confirmed
20Y513C1901	Poly A Tail Length Confirmed
21Y513C2201	Poly A Tail Length Confirmed
21Y513C2501	Poly A Tail Length Confirmed

Table 3.2.S.4.5-19. Commercial Release Acceptance Criterion for Poly(A) Tail Length

Quality Attribute	Acceptance Criterion
Poly(A) Tail Length	Poly(A) Tail Length Confirmed

Poly(A) Tail content (SO2c)

The assessment of drug substance for presence of the RNA poly(A) tail as determined by droplet digital PCR (ddPCR) is performed at release and on stability. The presence of the poly(A) tail protects the RNA thereby helping to ensure translation. The RNA species containing poly(A) tail are quantified as a percentage by assessing the poly(A) tail copy number from the total number of RNA copies in the sample. Data for process 1 DS obtained during development are shown in Table 3.2.S.4.5-15. Release data for forty-two process 2 commercial / emergency supply (EUA) DS batches are shown in Table 3.2.S.4.5-26 (not included in this report).

Table 3.2.S.4.5-15. Development, Comparability and Release Data for Poly(A) Tail (%)

BNTb2 Drug Substance Batch	Poly(A) Tail (%)
R427-P020.2-DS[a]	[redacted]
R438-P020.2-DS [a]	
R443-P020.2-DS [a]	
R445-P020.2-DS [a]	

a. Data obtained using a development method
b. Data in parentheses obtained during comparability assessment (Section 3.2.S.2.6 Development History and Comparability Assessment) using method performed for process 2 drug substance.

To establish the acceptance criterion lower limit for poly(A) tail, data from all batches manufactured by process 1 and process 2 have been considered. The comparability of these DS materials has been evaluated, as shown in Section 3.2.S.2.6 Development History and Comparability Assessment, and process 1 and 2 DS batches were demonstrated to be comparable.

Although comparability was established between process 1 and process 2 batches for this attribute, statistical assessment of the process 2 (EUA/Commercial) batch release data was performed on the process 2 DS data set only to determine a [redacted] for this attribute. Table 3.2.S.4.5-16 contains a summary of the assessment of the process 2 (EUA/Commercial Supply) batches, including the calculation of a lower limit for the poly(A) tail attribute based on [redacted] Data for Clinical (Process 1) batches obtained during comparability testing shown for information only.

Table 3.2.S.4.5-16. BNT162b2 Drug Substance Poly(A) Tail (%) Release Data: Statistical Analysis

Description	Clinical	EUA/Commercial Supply (All)	EUA/Commercial Supply (All, set >100 = 100%)
N (# of batches):	[redacted]		
Mean:			
SD:			
Min, Max:			
[redacted]			

a. For comparison, clinical batch data generated during comparability using the same method as for process 2 DS testing were used, rather than data from the development method.
b. NA: statistical treatment not appropriate for this size data set. Data shown in parenthesis for comparison only

There is no apparent change in the level of poly(A) tail in DS when stored at -20 °C, the recommended condition. The data up through 6 months for PPQ batches 20E162001, 20E162002, 20E162003, 20Y513C501 to 701 shown in Section 3.2.S.7.3 Long Term suggest that any appearance of an upward trend is likely related to method variability. Acknowledging that the poly(A) tail assay produces values greater than 100%, the process 2 data were further analyzed by setting all release values >100% equal to 100%. The calculated lower limit for this treatment [redacted] was essentially the same as the current specification [redacted]. Based on this understanding, the proposed acceptance criterion for commercial release and stability for poly(A) tail is presented in Table 3.2.S.4.5-17 as [redacted].

5'- Cap

Reverse phase HPLC (RP-HPLC) with UV detection is used to evaluate the relative amount of 5'-capped RNA species in BNT162b2 drug substance. The 5'- cap assay is performed at release and during stability studies.

Preliminary data obtained using a development method for process 1 batches (R427-P020.2-DS through R445-P020.2-DS) as well as results obtained during comparability assessment for a subset of these batches are shown in Table 3.2.S.4.5-12.

Table 3.2.S.4.5-12. BNT162b2 Development, Comparability and Release Data for 5'-Cap (%)

BNTb2 Drug Substance Batch	5'- Cap (%)
R427-P020.2-DS[a]	[redacted]
R438-P020.2-DS [a]	
R443-P020.2-DS [a]	
R445-P020.2-DS [a]	

a. Data obtained using a development method.
b. Data in parentheses obtained during comparability assessment (Section 3.2.S.2.6 Development History and Comparability Assessment) using method performed for process 2 drug substance.

To establish the acceptance criterion lower limit for 5'- cap, data from all batches manufactured by process 1 and process 2 have been considered. Drug product lots manufactured from the process 1 drug substance batches have been assessed clinically.

While 5'-cap data from process 1 and process 2 batches have been considered, with the implementation of process 2 manufacture there has been an increase in the 5'-cap percentage obtained. As a result, the statistical assessment of the 5'- cap data was performed on the process 2 (EUA/Commercial) drug substance data set only. Table 3.2.S.4.5-13 contains a summary of the assessment of the process 2 (EUA/Commercial) batches, including the calculation of a lower limit for the 5'-cap attribute based on [redacted]

Table 3.2.S.4.5-13. BNT162b2 Drug Substance 5'-Cap (%) Release Data: Statistical Analysis

Description	Clinical	EUA/Commercial Supply (All)
N (# of batches):	[redacted]	
Mean:		
SD:		
Min, Max:		
[redacted]		

a. For comparison, clinical batch data generated during comparability using the same method as for process 2 DS testing were used, rather than data from the development method.
b. NA: statistical treatment not appropriate for this size data set. Data shown in parenthesis for comparison only.

There is no significant change in the level of 5'-cap in drug substance when stored at -20 °C, the recommended condition, for up to 6 months. Consequently, and understanding that the immunogenicity of the drug product is correlated with antigen expression, which is in turn a function of successful translation of the RNA, the acceptance criterion (lower limit) for the proposed commercial release and stability for 5'-cap has been increased from ≥ 75% to ≥ 78%, as presented in Table 3.2.S.4.5-14. Drug substance with a value as low as 56% 5'-cap has been used for manufacture of drug product shown to be efficacious in clinical studies.

Table 3.2.S.4.5-14. Commercial Release and Stability Acceptance Criterion for 5'-Cap

Quality Attribute	Acceptance Criterion
5'-Cap	[redacted]

RNA integrity

Capillary gel electrophoresis is routinely used to evaluate the RNA integrity of drug substance at release and during stability. The method can detect potential degradation products based on their respective migration times and data are reported as relative peak area. Changes were made in the drug substance method related to data processing (See Section 3.2.S.2 6 Analytical Method Evolution for details and bridging information) in preparation for process 2 manufacture. Bridging studies demonstrated that data processed by the updated processing method resulted in apparently lower % intact RNA results, with a systematic offset of as much as [redacted]. See Table 3.2.S.4.5-9 for comparative results pre- and post-reprocessing for nonclinical and process 1 drug substance batches (where batch R427-P020.2-DS shows an offset of [redacted]. (Note that the certificate of analysis results for nonclinical and process 1 drug substance batches correspond to results obtained prior to reprocessing). The comprehensive release test results for RNA integrity for forty-two BNT162b2 drug substance batches manufactured for commercial and emergency supply using process 2 are shown in Table 3.2.S.4.5-26; these data were obtained using the updated data processing procedure.

Table 3.2.S.4.5-9. BNT162b2 Process 1 (Nonclinical and Clinical) Drug Substance Release Data for RNA Integrity

Batch	RNA Integrity (%)	
	Result from Raw Data Reprocessing [a]	*Certificate of Analysis Result*
RNA-RF200321-06	[redacted]	[redacted]
R427-P020.2-DS		
R438-P020.2-DS		
R443-P020.2-DS		
R445-P020.2-DS		

a. Result obtained by reprocessing raw data (see Section 3.2.S.2.6 Analytical Method Evolution). Reprocessing used updated processing method employed for process 2/commercial release testing.

The acceptance criterion for intact RNA was previously established in conjunction with process 2 DS manufacture and in light of the demonstrated comparability of process 1 and process 2 materials and set at [redacted], based on the release testing results for clinical process 1 batches.

To establish the reassessed acceptance criterion for intact RNA, data from all batches manufactured by process 1 and process 2 as shown in Table 3.2.S.4.5-26 (R427-P020.2-DS through R445-P020.2-DS (reprocessed data), 20Y513C101 through 20Y513C2501, and 20E162001 through 20E162019) have been considered. To derive the commercial acceptance criterion for DS RNA integrity, data were evaluated statistically. Because results from DS manufacturing process characterization studies suggested that by increasing the concentration of specific nucleotide triphosphates somewhat higher production yields and potentially higher % integrity may be achieved, as demonstrated by the integrity results from 20Y513C501 and more recent DS batches, a statistical assessment was performed on both the RNA integrity results for the forty-two process 2 batches, as well as on the integrity results of the process 2 batches excluding those that were manufactured prior to the nucleotide triphosphate adjustment (38 batches starting with 20Y513C501, see Table 3.2.S.4.5-26) to determine a one-sided limit (mean – 3SD). The results of these assessments, as well as comparison to process 1 clinical drug substance batch results (R427, R438, R443, and R445), are presented in Table 3.2.S.4.5-10.

Table 3.2.S.4.5-10. BNT162b2 Drug Substance Release Data for RNA Integrity: Statistical Assessment

Description	Clinical	EUA/Commercial Supply (All)	EUA/Commercial Supply (minus early process 2)
N (# of batches):		[redacted]	
Mean:			
SD:			
Min, Max:			
[redacted]			

a. NA: statistical treatment not appropriate for this size data set. Data shown in parenthesis for comparison only.

Process 2 drug substance batches (EUA/Commercial) demonstrate a range of RNA integrity release testing results from [redacted] with a calculated [redacted] limit of [redacted] When the early (pre-nucleotide optimization batches, 20Y513C101 to 20Y513C401) are excluded from the process 2 drug substance data set, the lower end of the observed range as well as the [redacted] lower limit ([redacted]) increase. While the range of RNA integrity release data for the clinical process 1 batches ([redacted]) is higher than for the process 2 batches, the activity of the product is dependent on a combination of RNA attributes in addition to integrity, most notably the presence of a 5'- cap. The 5'- cap critical quality attribute is discussed in Section 3.2.S.4.5.9. The slightly lower process 2 drug substance RNA integrity relative to process 1 is off-set by the increased level of 5'-cap in process 2 drug substance relative to that in process 1.

There is no significant trend in RNA integrity for representative batches of drug substance stored at the recommended condition of -20 ± 5 °C for up to 6 months. (See Section 3.2.S.7.3 Long Term and Section 3.2.S.7.1 Stability Summary and Conclusions.) As a result, no separate stability acceptance criterion for RNA integrity in drug substance related to storage at recommended conditions is required.

In addition to the understanding gained by DS manufacturing characterization, additional experience at commercial scale and associated characterization of the DP manufacturing suggests that an allowance for some potential loss in RNA integrity across the DP formulation and fill/finish processes may be needed to ensure appropriate RNA integrity in DP at release and throughout shelf life, including at point of administration. Consequently, and based on this understanding as well as on the determination of the lower limit based on [redacted] as calculated from the post nucleotide adjustment process 2 drug substance release data, the acceptance criterion (lower limit) for commercial release and stability for RNA integrity in DS has been tightened to ≥ 68%, as is shown in Table 3.2.S.4.5-11. This value would allow for an up to 10% drop in integrity, that could result from drug product manufacturing operations, while meeting the appropriate quality attribute level of ≥58% RNA integrity at release in drug product.

Table 3.2.S.4.5-11. Commercial Release and Stability Acceptance Criterion for RNA Integrity

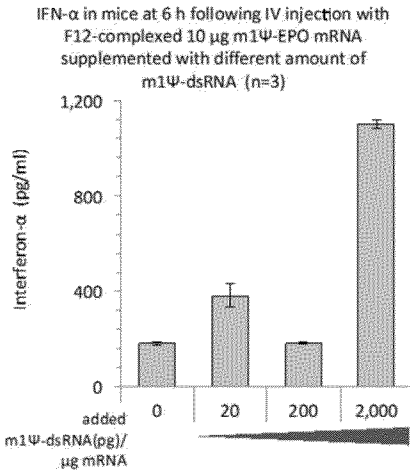
Test	Acceptance Criterion
Capillary Gel Electrophoresis	[redacted] intact RNA

dsRNA - Immunoblot

Double stranded RNA (dsRNA), a product-related impurity in BNT162b2 drug substance, is determined by immunoblot analysis. It is measured for every batch of BNT162b2 drug substance at release.

When dsRNA enters into the endosome or cytoplasm, cells sense it as viral invader. All cells are capable to respond to such dsRNA through various pattern recognition receptors (e.g.TLR3, RIG-I, MDA5) and effectors (e.g. OAS, PKR)1. However, the expression pattern of these receptors and effectors is cell-type specific. Stimulation of the dsRNA sensors leads to secretion of different cytokines, including type-I interferon, IL-6, and TNF-α. Thus, controlling the level of dsRNA in in vitro transcribed mRNA is important to limit induction of cytokines. To understand at what level this might become critical, a mouse study was performed, where m1Ψ-modified mRNA encoding erythropoietin (EPO) containing different amounts of dsRNA (0 – 2000 pg/µg mRNA) was formulated and injected intravenously (i.v.) into mice. Cytokine measurements revealed background levels of IFN-α in mice dosed with up to 200 pg dsRNA/µg mRNA; only at the 2000 pg dsRNA/µg mRNA was an increased level of IFN-α observed (Figure 3.2.S.4.5-1).

Figure 3.2.S.4.5-1. Interferon alpha (IFN-α) Levels Following injection of m1Ψ-dsRNA



Results of i.v. injection of formulated EPO mRNA containing different amounts of dsRNA into mice. Interferon-α secretion upon transfection of 10 µg EPO mRNA.

The data above were related to the human dose of BNT162b2, 30 µg RNA, by calculation of the dsRNA dose per respective body weight (pg/kg) and conversion into dsRNA dose per body surface area (pg/m²), which is the parameter recommended for comparing animal models and human subjects. The results of this comparison are as follows:

First, the calculation of the dsRNA dose per body surface area, based on the of 10 µg dose used in the mouse experiments, shows that dsRNA doses of ≤ 300,000 pg/m² (corresponding to ≤ 200 pg dsRNA/ μ g RNA) do not affect IFN- α secretion.

Second, the relationship of the mouse data to human dosing was assessed by calculating the maximal dose per body surface area using the proposed acceptance criterion (500 pg dsRNA/µg) for two cases: an adult with a body weight of 60 kg and an infant with a body weight of 5.6 kg. The calculated dose of dsRNA per body surface area was in each case below 300,000 pg/m² as determined from the mouse study (Table 3.2.S.4.5-22), with the highest calculated dose per body surface area (99,107 pg/m2) for infants dosed with 30 µg and a body weight of 5.6 kg. This value is approximately 3-fold lower than the corresponding dose per body surface area mouse result at the highest tested NOEL (no observed effect level; 300,000 pg/m2).

Table 3.2.S.4.5-22. Conversion of dsRNA Acceptace Criteria (pg/µg RNA) into dsRNA per Body Surface Area (pg/m²)

	Human, i.m. injection of m1Ψ-COMIRNATY mRNA [adult]	Human infant, i.m. injection of m1Ψ-COMIRNATY mRNA [infant]
dsRNA (pg/µg RNA)	[redacted]	
Dose (µg)		
Standard body weight (kg)[a, b]		
Dose/weight (pg/kg)		
Conversion factor k_m[a, b]		
Dose/body surface area (pg/m²)[a, b]		

a. Nair and Jacob[2]
b. FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (July 2005)

The proposed acceptance criterion of [redacted] pg dsRNA/ μ g RNA provides an approximate [redacted] factor (over the lowest dose per body surface area calculation in humans) to minimize the unspecific immunological effect and is aligned with the capability and validation of the optimized immunoblot limit test. Based on these considerations the commercial acceptance criterion for dsRNA at release is presented in Table 3.2.S.4.5-23.

Table 3.2.S.4.5-23. Commercial Release Acceptance Criterion for dsRNA

Quality Attribute	Acceptance Criterion
Residual dsRNA	[redacted]g dsRNA/µg RNA

Assessor´s comments on S.4.5:

Poly(A) Tail Length

New method with acceptance criterion added.

Poly(A) Tail content (SO2c)

Poly(A) tail content determination by the ddPCR method is a two-step process, involving reverse transcription of mRNA into cDNA followed by digital droplet quantitation of the cDNA. A method assessment was undertaken to identify potential causes of poly(A) content overestimation, and the method suitability was re-evaluated in the context of these potential causes of overestimation.

Over-primming of OligoDT22VN was identified as one of possible causes, in the event the reverse transcription primer anneals to both the 30 and the 70 adenosines segments of BNT162b2. A second potential cause of poly(A) tail content over-estimation is the presence of fragments derived from terminated transcripts as the poly(A) content is calculated by dividing the measured poly(A) tail copies/µL (from the ddPCR assay) by the input number of mRNA copies (targeting 1000 copies/µL), which might be biased by the presence of the prematurely terminated transcripts in the sample.

The consistency of the method has been investigated by analysis of an internal control sample over 104 instances. The average poly(A) content by ddPCR was observed to be [redacted], based on which it was concluded that the consistent performance of the method is demonstrated. Although this is agreed with, the possible impact of over-primming on the accuracy of the method has not been addressed by this study. It can be speculated that, although the method performs in a consistent way, it might still overestimate (consistently) the presence of poly(A) tails in the samples. It would have been of value if these analyses also would have been performed using samples with high and low content of poly(A) tail (ranging from [redacted] Table 3.2.S.4.5-26). In view of the data presented now,

indicating consistency in over-estimation, and the relatively high number of batches (9 out 42) with poly(A) content in the range of [redacted], the accuracy of the method is questioned, and it needs to be further addressed.

The fragment species are not considered to contribute to the overestimation of poly(A) tail as the fragment species content has remained consistent throughout the manufacturing history, and the attribute is controlled through specification acceptance criteria for RNA integrity.

- The accuracy of the ddPCR method needs to be further addressed in relation to available data indicating a consistent overestimation of % poly(A) tail content, as 9 out 42 batches provided show results in the range of [redacted].

dsRNA

Tightened acceptance criterion from [redacted] dsRNA/ µg RNA to [redacted] dsRNA/ µg RNA. This change was approved in EMEA/H/C/005735/II/045.

The limit is now further justified based on results from a mouse study. Cytokine measurements revealed background levels of IFN-α in mice dosed with up to [redacted] dsRNA/µg mRNA; only at the [redacted] dsRNA/µg mRNA was an increased level of IFN-α observed. The proposed acceptance criterion provides an approximate [redacted] (over the lowest dose per body surface area calculation in humans) to minimize the unspecific immunological effect. The justification presented is found acceptable.

5'-Cap

For the 5'-Cap attribute, additional data, based on statistical analysis of 42 EUA/Commercial Supply batches, have been provided, with a min/max range of [redacted] 5'-Cap levels and a [redacted] of [redacted] which takes into account the manufacturing capacity of the implemented process 2 and method variability. To be noted, the improvement implemented in the manufacturing process, from process 1 to process 2, resulted in a significantly increase in the 5'-cap percentage obtained for the EUA/Commercial Supply batches. The proposed specification is now increased from [redacted] which does not take into account process capability. However, as the proposed limit is based on early clinical batches with 5'-Cap levels of [redacted] which have been previously demonstrated to be efficacious, this issue is not further pursued. In line with GMP requirements the company is expected to evaluate any out of trends results.

RNA integrity

The acceptance criterion for RNA integrity was tightened form [redacted] and approved in connection with EMEA/H/C/005735/IB/0031/G (partial fulfillment of SO2a, SO2d). No further tightening is proposed or considered necessary.

Conclusion:

For Poly (A) tail length a new method with a qualitative acceptance criterion has been added and for 5'-Cap the criterion has been tightened from [redacted] these are the only changes made to the DS specification.

SO2a is considered solved from a DS perspective.

6.1.4. Stability (3.2.S.7)

For drug substance batch EP3345, the RNA integrity acceptance criterion has been updated from [redacted] to reflect the revised specification implemented at the 3 month time point. This change has been updated in Section 3.2.S.7.3 Long-Term and Section 3.2.S.7.3 Accelerated.

For drug substance batches 20E162001, 20E162002, and 20E162003 stored at -80 to -60 °C and at 25±2 °C, the RNA integrity acceptance criterion has been updated from [redacted] to reflect the revised specification implemented at the 1 month time point. These changes have been updated in Section 3.2.S.7.3 Thermal Stress.

To date, twelve PPQ drug substance batches, MB0001, MB0002, MB0003, EP3345, 20Y513C301, 20Y513C401, 20Y513C501, 20Y513C601,20Y513C701, 20E162001, 20E162002, and 20E162003 manufactured using the commercial process (Process 2) from the manufacturing sites Pfizer Andover, BioNTech Mainz/Rentschler and BioNTech Marburg have been placed on stability and stored under long term, accelerated, and thermal stress conditions.

A summary of all drug substance batches on stability studies and current available stability data are shown in the dossier.

The current shelf life for the BNT162b2 drug substance is 6 months when stored at the recommended temperature of -20 ± 5 °C in EVA bags.

Assessor´s comments:

The applicant has provided updated drug substance stability data (including additional batches/lots and data at additional stability study time points) in support of the updated specifications. This updates are found acceptable.

The current shelf life of the drug substance, 6 months when stored at -20 ± 5 °C remains unchanged.

6.2. Drug product

6.2.1. Control of Drug Product (3.2.P.5)

6.2.1.1. Drug Product Specifications (3.2.P.5.1)

The specification for BNT162b2 drug product at release and throughout shelf life is provided in Table 3.2.P.5.1-1. The acceptance criteria for quality attributes tested both at release and on stability are the same with the exception of RNA integrity and LNP size, for which separate stability and release acceptance criteria have been established.

Table 3.2.P.5.1-1. BNT162b2 Drug Product Specification

Quality Attribute	Analytical Procedure[a]	Procedure Number(s)	Acceptance Criteria
Composition and Strength			
Appearance	Appearance (Visual)	TM100010539 [g] TM9002A [j] SOP-10173 [l] A-Q-082 [n] 10032.01 [p]	White to off-white suspension
Appearance (Visible Particulates)	Appearance (Particles)	Ph Eur. 2.9.20, USP <790>, JP 6.06	May contain white to off white opaque, amorphous particles
Subvisible Particles	Subvisible Particulate Matter *(USP <787>, light obscuration method)*	TM100010541 [g] SOP-13114 [i] PV-Q-1279 [n] QK TM 47872e [o] 37301.01 [p]	[redacted]
pH	Potentiometry	Ph. Eur. 2.2.3, USP <791>	
Osmolality	Osmometry [b,c] *(USP <785>)*	TM100010540 [g] TM8209A [j] SOP 7040133 [l] QK TM 47872e [o] 13711.01 [p]	
LNP Size	Dynamic Light Scattering (DLS)	TM100010649 [g] PV-Q-1270 [n] SOP-10021 [l] TM9119A [j] PAN-1288-K [m]	
LNP Polydispersity	Dynamic Light Scattering (DLS)	TM100010649 [g] PV-Q-1270 [n] SOP-10021 [l] TM9119A [j] PAN-1288-K [m]	
RNA Encapsulation	Fluorescence assay	TM100011182 [g] PV-Q-1272 [n] SOP-10013 [l] TM9130A [j] PAN-1331-K [m]	
RNA content	Fluorescence assay	TM100011182 [g] PV-Q-1272 [n] SOP-10013 [l] TM9130A [j] PAN-1331-K [m]	

Table 3.2.P.5.1-1. BNT162b2 Drug Product Specification

Quality Attribute	Analytical Procedure[a]	Procedure Number(s)	Acceptance Criteria
ALC-0315 content ALC-0159 content DSPC content Cholesterol content	HPLC-CAD HPLC-ELSD[e]	TM100010322 [g] SOP-10186 [l] PV-Q-1269 [n] TM8891A [j] PAN-1287-K [m]	[redacted]
Vial content (volume)	Container content[c]	TM100011129 [g] TM9125A [j] QK TM 47872e [o] 12301.01 [p]	
Identity			
Lipid identities	HPLC-CAD[c] HPLC-ELSD [c, e]	TM100010322 [g] PV-Q-1269 [n] SOP-10186 [l] TM8891A [j] PAN-1287-K [m]	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)
Identity of encoded RNA sequence	RT-PCR[c]	TM100010407 [g] SOP-111956 [i] PAN-1235-K [m] TM-072-038 [k] LAB-37698 [h]	Identity confirmed
Potency			
In Vitro Expression	Cell-based flow cytometry	TM100010380 [g] SOP-113198 [i] PAN-1215-K [m] PAN-1216-K [m] LAB-38621 [h]	[redacted]
Purity			
RNA Integrity	Capillary Gel Electrophoresis	TM100010392 [g] PAN-1234-K [m] PV-Q-1271 [n] TM9089A [j] TM-072-039 [k]	[redacted] (stability)
Adventitious Agents			
Bacterial Endotoxin	Endotoxin (LAL)	Ph. Eur. 2.6.14, USP <85>, JP 4.0	≤ 12.5 EU/mL
Sterility	Sterility	Ph. Eur. 2.6.1, USP <71>, JP 4.06 LAB-37663 [f, j]	No Growth Detected

Table 3.2.P.5.1-1. BNT162b2 Drug Product Specification

Quality Attribute	Analytical Procedure[a]	Procedure Number(s)	Acceptance Criteria
Container Closure Integrity	Dye incursion[d]	TM100010635 [g] PV-Q-1280 [n] QKM TM 47847e [o]	Pass

a. All assays performed on stability unless otherwise noted.
b. In accordance with Ph. Eur. 2.2.35, with minor difference in instrument calibration
c. Assay not performed on stability.
d. Tested at release and on stability for stability lots only
e. Test used at mibe instead of HPLC-CAD
f. Rapid Sterility Test, which is performed in accordance with the compendia with the exception of incubation duration and detection method (see Section 3.2.P.5.2 Sterility), may also be used.
g. Analytical procedure at Pfizer, Analytical Research and Development
h. Analytical procedure at Pfizer Global Supply, Andover, MA, USA
i. Analytical procedure at Pfizer Global Supply, Grange Castle, Ireland
j. Analytical procedure at Pfizer Global Supply, Puurs, Belgium
k. Analytical procedure at BioNTech, Mainz
l. Analytical procedure at BioNTech, Marburg
m. Analytical procedure at BioNTech IMFS
n. Analytical procedure at mibe
o. Analytical procedure at Baxter (for appearance (visual) no test method is in place)
p. Analytical procedure at Novartis

Abbreviations: LNP = Lipid nanoparticles; CAD = charged aerosol detector; ELSD = evaporative light scattering detector; RT-PCR = reverse transcription polymerase chain reaction; FACS = fluorescence activated cell sorter; ddPCR = droplet digital PCR; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amebocyte lysate; EU = endotoxin unit

6.2.1.2. Justification of Specifications (3.2.P.5.6)

Introduction

The specification for BNT162b2 drug product is based on an understanding of the control strategy and CQAs for the drug product. The attributes tested and associated acceptance criteria ensure the consistency of drug product and linkage to clinical experience. This specification was established to ensure the quality, purity, potency/biological activity and safety of the commercial drug product at release and during storage. The specification was informed by:

-Development experience (manufacture and analytical) with and data for BNT162b2 drug product, as well as modRNA platform knowledge

-Total BNT162b2 manufacturing experience, including drug product lots used in development, nonclinical and clinical studies, process characterization studies and process performance qualification, and manufacturing for commercial supply

-The release and ongoing stability data for drug product 3.2.P.5.6.2. Specification Setting Strategy

A comprehensive panel of analytical procedures was implemented along with corresponding acceptance criteria (Section 3.2.P.5.1 Specification as well as this section) to monitor and control BNT162b2 drug product quality at release and during shelf life.

Appropriate analytical procedures were established to monitor and assess BNT162b2 drug product as detailed in Section 3.2.P.5.2 Analytical Procedures and Section 3.2.P.5.3 Validation of Analytical Procedures. With the exception of osmometry (osmolality), volume of injections in containers (container content for injections), HPLC-CAD (lipid identities), and RT-PCR (identity of encoded RNA sequence) assays, which are conducted at drug product release only, all other procedures are conducted at release

and during stability studies for drug product. The container closure integrity test is used only for the stability studies.

The approach to setting acceptance criteria for each quality attribute in the BNT162b2 drug product specification included understanding gained from:

-Data obtained for drug product lots used as nonclinical toxicology and clinical trial supplies, as well as data obtained from lots used for commercial supply and emergency supply (other markets).

-Manufacturing experience and knowledge of process capability and consistency determined based on full-scale drug product release data, including data from process qualification and validation batches.

-The relevant long-term stability data that were obtained for the BNT162b2 drug product at recommended storage conditions of -90 °C to -60 °C (Section 3.2.P.8.1 Stability Summary and Conclusion and Section 3.2.P.8.3 Stability Data – Long-Term).

-Experience with the analytical procedure and knowledge of the method capabilities.

-Comparability demonstrated across the development history. See Section 3.2.P.2.3 Development History and Section 3.2.P.2.3 Comparability Assessment of PPQ Lots.

-The regulatory expectations for RNA-based products, where appropriate.

-Relevant BNT162b2 development data, available literature, and the institutional experience with other mRNA products.

Testing results for each of the quality attributes obtained for drug product lots used as clinical trial supplies served as the basis for clinical justification of the specifications.

Based on stability data for representative BNT162b2 drug product at the recommended storage condition of -90 to -60 °C, the acceptance criteria used for stability during shelf life is predominantly the same as the acceptance criteria used for lot release, with the exception of the LNP size and RNA integrity attributes for which a separate stability acceptance criterion has been established to enable alternate storage at -20 °C and 2 -8 °C at the point of administration, which is critical to enable vaccine access and mass vaccination. The acceptance criteria in the drug product specification reflect the current understanding of criticality of quality attributes, their impact on product performance, and the quality of the product used in clinical trials to ensure consistent manufacture of drug product.

A global approach to development has been undertaken across multiple manufacturing facilities in order to maximize vaccine production and availability. As such, information and analysis presented within Section 3.2.P.5.6 Justification of Specification(s) section reflects the global development effort and the drug product lots included in the specification analysis are not limited to those produced in market-specific registered manufacturing facilities. The lots included in the evaluation and establishment of the commercial specification are presented in Table 3.2.P.5.6-1. The lots shown represent one nonclinical toxicology lot, four clinical lots/lots designated for clinical use (BCV40420, BCV40620, BCV40720 and EE3813) including sublots (A,B, etc.) as appropriate and manufactured from process 1 drug substance, and eighty-three commercial scale drug product lots manufactured from process 2 drug substance, including fourteen process performance qualification lots (seven manufactured at the Pfizer, Kalamazoo facility and seven manufactured at the Pfizer, Puurs site). Drug product lots corresponding to process performance qualification lots are identified in Table 3.2.P.5.6-1.

Due to the size of the data set, all numerical release testing results (except for container content and endotoxin) have been summarized in Table 3.2.P.5.6-34 and Table 3.2.P.5.6-35.

Statistical Analysis

Statistical analysis was applied to the release data for the eighty-three commercial scale drug product lots manufactured between August 2020 and January 2021 (Section 3.2.P.5.4 Batch Analysis), and the mean, standard deviation and ± k*standard deviations from the mean for the release data set were calculated for each quality attribute, when applicable. A similar analysis for RNA encapsulation, in vitro expression, and RNA integrity was applied, and a one-sided limit with either plus or minus k*standard deviations was calculated. The k factor was chosen based on desired confidence and coverage. While additional commercial supply/emergency supply drug product lots have been manufactured more recently and additional manufacturing sites have been added, the data set contains all process performance qualification lots from the two initial fill/finish manufacturing facilities as well as from the lipid nanoparticle manufacturers, and a significant number of total lots, thereby providing a robust data set for statistical analyses.

Once the statistical analysis results were generated for each of the quality attributes, the acceptance criteria were further adjusted and justified based on various factors including the assessment against the clinical exposure range(s) determined from those drug product lots evaluated clinically; any available structure/function information; process capability determined through development and process validation; manufacturing experience; and experience with the analytical procedure, when applicable.

For those attributes not subjected to statistical analysis, acceptance criteria were determined based on understanding of formulation robustness, compendial requirements, clinical experience and/or literature references.

Table 3.2.P.5.6-1. BNT162b2 Drug Product Lots and Drug Substance Batches for Establishing the Specifications

Drug Product Lot Number	Drug Substance Batch Number	Purpose of Material
COVVAC/270320	RNA-RF200321-06	Nonclinical toxicology, Stability
BCV40420-A	R427-P020.2-DS	Clinical, Stability
BCV40620-A	R438-P020.2-DS	Clinical, Stability
BCV40620-B		Clinical
BCV40620-C		Clinical
BCV40620-D		Clinical
BCV40620-E		Nonclinical, Stability
BCV40720-A	R443-P020.2-DS	Clinical, Stability
BCV40720-B		Clinical
BCV40720-C		Clinical, stability
ED3938[a]		Clinical, Stability
EE3813[b]	R445-P020.2-DS	Clinical, Stability
EE8492[c]	20Y513C101	Commercial Supply / Emergency Supply[d], Stability
EE8493		Commercial Supply / Emergency Supply, Clinical, Stability
EJ0553	20Y513C501	Commercial Supply / Emergency Supply, Clinical, Stability
EJ0724	20E162001 (1071539)	Commercial Supply / Emergency Supply
EJ1685	20E162001 (1071539)	Commercial Supply/ Emergency Supply, Clinical Inventory, Stability
EJ1686	20E162001 (1071539)	
EH9899	20Y513C201	Commercial Supply / Emergency Supply, Stability
EJ1688	20E162002 (1071542)	
EK4175	20E162002 (1071542)	Commercial Supply / Emergency Supply
EK4176	20E162002 (1071542)	

Table 3.2.P.5.6-1. BNT162b2 Drug Product Lots and Drug Substance Batches for Establishing the Specifications

Drug Product Lot Number	Drug Substance Batch Number	Purpose of Material
EK1768	20Y513C401	Commercial Supply / Emergency Supply, Clinical Inventory, Stability
EK5730	20Y513C601	Commercial Supply / Emergency Supply
EL0140	20E162004 (1071545)	
EL0141	20E162003 (1071544)	
EL0142	20E162004 (1071545)	
EL0725	20E162003 (1071544)	
EL0739	20E162003 (1071544)	
EL1484	20E162003 (1071544)	
EK9231	20Y513C301	
EK4237	20E162005 (1071546)	
EK4243	20E162005 (1071546)	
EK4244	20E162006 (1071547)	
EL1283	20Y513C801	
EJ6795	20Y513C601	
EK4241	20E162003 (1071544)	
EK4245	20E162006 (1071547)	
EJ6796	20Y513C701	
EJ6797	20Y513C701	
EK4238	20E162004 (1071545)	
EK4240	20E162007 (1071548)	
EK4242[e]	20E162007 (1071548)	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EL1284	20Y513C601	Commercial Supply / Emergency Supply
EL7834[f]	20E162005 (1071546)	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EL1491[g]	20E162001 (1071539)	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Stability
EL3246	20Y513C701	Commercial Supply / Emergency Supply
EJ3002	20E162009 (1071552)	
EL0200	20E162008 (1071551)	
EL0203	20E162008 (1071551)	
EM0477	20E162002 (1071542)	
EL3248[h]	20Y513C501	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Stability
EJ6134	20E162001 (1071539)	Commercial Supply / Emergency Supply
EJ6136	20E162004 (1071545)	
EJ6788	20E162004 (1071545)	
EL1404	20E162008 (1071551)	
EL3249	20Y513C601	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Stability

Table 3.2.P.5.6-1. BNT162b2 Drug Product Lots and Drug Substance Batches for Establishing the Specifications

Drug Product Lot Number	Drug Substance Batch Number	Purpose of Material
EK9788	20E162005 (1071546)	Commercial Supply / Emergency Supply
EL1406	20E162008 (1071551)	
EN3924	20E162008 (1071551)	
EL3247	20Y513C1101	
EJ6789	20E162005 (1071546)	
EL3302	20Y513C1101	
EL8982	20Y513C1101	
EJ6790	20E162004 (1071545)	
EL8723	20Y513C801	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Stability
EM6950	20Y513C501	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EL9261	20Y513C1201	Commercial Supply / Emergency Supply
EL9262	20Y513C1201	
EN1185	20E162014 (1071557)	
EL9263	20Y513C1201	
EN9581	20Y513C1201	
EN5318	20Y513C1301	
EL9266	20Y513C1301	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EL9265	20Y513C501	Commercial Supply / Emergency Supply
EL8713	20E162002 (1071542)	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EP2163	20Y513C1101 20Y513C1401	Commercial Supply / Emergency Supply, Process performance qualification
EP2166	20Y513C1301	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EL9267	20Y513C601	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EL9269	20Y513C801	Commercial Supply / Emergency Supply
EL9264	20Y513C1401	
EM9809	20Y513C1401	
EM9810	20Y513C1501	
EP6775	20Y513C1201	Commercial Supply / Emergency Supply, Process performance qualification
EN6200	20Y513C1501	Commercial Supply / Emergency Supply, Process performance qualification
EN6201	20Y513C1401	Commercial Supply / Emergency Supply
EN1195*	20E162009 (1071552)	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EP6017	20Y513C1101	Commercial Supply / Emergency Supply
EP9598	20E162006 (1071547)	Commercial Supply / Emergency Supply
EN6198	20Y513C701 20Y513C801	Commercial Supply / Emergency Supply, Process performance qualification
EP9605	20E162006 (1071547)	Commercial Supply / Emergency Supply
EN1196*	20E162015 (1071558)	Commercial Supply / Emergency Supply, Process performance qualification

Table 3.2.P.5.6-1. BNT162b2 Drug Product Lots and Drug Substance Batches for Establishing the Specifications

Drug Product Lot Number	Drug Substance Batch Number	Purpose of Material
EN6199	20Y513C1501 20Y513C1601	Commercial Supply / Emergency Supply, Process performance qualification, Stability
EM4965[f]	20E162016 (1071559) 20E162017 (1071560)	Commercial Supply / Emergency Supply, Process performance qualification, Stability
ET0384[f]	20Y513C1601	Commercial Supply / Emergency Supply, Process performance qualification

a. This lot number is equivalent to BCV40720-P, a sublot of BCV40720.
b. This lot number is equivalent to BCV40820-P.
c. Commercial scale lot from here forward
d. Emergency supply designation applies to U.S. market
e. Corresponds to PPQ lot for which LNP was manufactured at mibe (also known as Dermapharm) and fill/finish at Pfizer, Puurs Belgium site.
f. Corresponds to PPQ lot for which LNP was manufactured at Polymun and fill/finish at Pfizer, Puurs Belgium site
g. Lots identified in bold, italicized text correspond to PPQ lots manufactured (LNP as well as fill/finish) at Pfizer, Puurs Belgium site
h. Lots identified in bold text correspond to PPQ lots manufactured (LNP, as well as fill/finish) at Pfizer, Kalamazoo site

Clinical Relevance

Acceptance criteria have been assessed relative to those lots that have been used clinically, as well as with respect to recently published evidence of efficacy associated with multiple early lots from the emergency supply. Since emergency authorization of the vaccine, Pfizer-BNT162b2 commercial supplies have been introduced and efficacy studied in a nationwide mass vaccination setting in Israel[1]. The lots supplied to Israel were reviewed regarding manufacturing site and process, as well as attribute results.

As reported by Dagan et al., persons newly vaccinated during the period from 12/20/2020 to 2/1/2021 were matched to unvaccinated controls and outcomes were monitored. Each study group included 596,618 persons. Efficacy of the vaccine was confirmed at 7 days or more after the second dose: 92% vaccine effectiveness for documented infection, 94% vaccine effectiveness for symptomatic Covid-19. "This study estimates a high effectiveness of the BNT162b2 vaccine for preventing symptomatic Covid-19, similar to the vaccine efficacy reported in the randomized trial."[1]

The COVID-19 vaccine lots that were shipped to Israel in December, 2020 through January 15, 2021 are listed in Table 3.2.P.5.6-2, along with the shipping date (goods movement date).

Additional lots have been shipped since January 15, 2021 but are not included in this list to be conservative in representing the lots that would be predicted to have been used in the mass vaccination setting in Israel, based on the timing defined in Dagan et al.[1]

The LNP size, LNP polydispersity, RNA encapsulation, RNA integrity (including the percent late migrating species (LMS) for additional information), and in vitro expression (IVE) results for these lots are also listed. It should be noted that all these lots utilized drug substance manufactured by Process 2 and were manufactured at commercial scale at mibe (Dermapharm) or Polymun for lipid nanoparticle (LNP) and filled at Puurs.

[1] Dagan Noa, Barda Noam, Kepten Eldad, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. New England Journal of Medicine 2021;384 15:1412-1423.

Table 3.2.P.5.6-2. Lots Supplied to Israel from Authorization until 15 Jan 2021

Lot	Goods Movement Date	LNP Size (nm)	LNP Poly-dispersity	RNA Encapsulation (%)	RNA Integrity (%)	LMS[a] (%)	IVE (% positive cells)	DP Manu-facturing Site(s)
EK4175	12/8/2020, 12/9/2020, 12/15/2020	[redacted]						DER[b]
EK4237	12/21/2020, 12/23/2020							DER
EK4241	12/21/2020							PLY[c]
EK4238	12/28/2020							PLY
EK4240	12/29/2020							DER
EL0200	12/30/2020							DER
EK4242	12/30/2020							DER
EJ3002	1/8/2021							PLY
EL0203	1/8/2021							DER
EL1404	1/15/2021							DER

a. LMS = Late Migrating Species
b. DER refers to LNP manufacture at mibe (Dermapharm) with fill/finish at Pfizer, Puurs
c. PLY refers to LNP manufacture at Polymun with fill/finish at Pfizer, Puurs

Osmolality

Osmolality performed according to USP <785>, and in accordance with Ph. Eur. 2.2.35 with slight difference in instrument calibration, is measured for every lot of BNT162b2 drug product for release.

The osmolality range for BNT162b2 drug product reflects the buffer and cryoprotectant matrix composition designed to provide long term stability at the current target storage condition of -90 °C to -60 °C (Section 3.2.P.2.2 Drug Product). The acceptance criterion is based on the osmolality expected due to the formulation components. Data for BNT162b2 drug product are shown in Table 3.2.P.5.6-34, and are summarized in Table 3.2.P.5.6-8 demonstrating a range in osmolality of [redacted] mOsmol/kg for clinical lots (n=10) and [redacted] mOsmol/kg for commercial / emergency supply drug product lots (n=83).

Table 3.2.P.5.6-8. BNT162b2 Drug Product Release Data Summary for Osmolality

Description	Clinical[a]	Commercial / Emergency Supply
N (# of lots):	10	83
Min, Max:	[redacted]	

a. Drug product manufactured from process 1 and used in clinical trials during development

The commercial acceptance criterion for osmolality at release is presented in Table 3.2.P.5.6-9.

Table 3.2.P.5.6-9. Commercial Release Acceptance Criterion for Osmolality

Quality Attribute	Acceptance Criterion
Osmolality	[redacted] mOsmol/kg

LNP Size

LNP size is measured for BNT162b2 drug product using dynamic light scattering (DLS). The efficacy of the drug product depends on the size of the LNP being controlled within a certain range. The LNP size must be both large enough to encapsulate RNA, as well as small enough to pass through the [redacted] sterile

filtration membrane during manufacture. Particle size is also correlated to product stability. Historical experience[2] with similar systems has indicated that well formed LNP are generally homogeneous with relatively narrow size distribution and a mean size that is within a preferred range, generally below [redacted] The preferred range is likely related to the mode of action involving the clathrin pathway[3].

Manufacturing experience has produced drug product with LNP size ranging from [redacted] (min to max) in the lots used for clinical studies during development (n=10) and from [redacted] in the commercial / emergency supply (n=83) at time of release. See Table 3.2.P.5.6-34 for the release data and Table 3.2.P.5.6-10 for a data summary. No trend in LNP size has been observed up to 6 months and 9 months at recommended storage (-90 °C to -60 °C) for commercial / emergency supply lots and for the clinical stability lots stored at -70 °C, respectively. An increase in LNP size for commercial / emergency supply drug product has been observed, however, at storage conditions of -20 °C and 5 °C, with one emergency supply lot (EE8493) and one PPQ lot (EK4242) outside of the proposed commercial specification when stored at -20 °C for 3 months (Section 3.2.P.8.3 Accelerated Stability Data and Section 3.2.P.8.1 Stability Summary and Conclusion).

The statistical assessment of the data to determine a two-sided acceptance range (mean ± 3SD) leads to a calculated range of [redacted] for the commercial / emergency use lots (n=83) and of [redacted] for the clinical lots (n=10). A summary of the comprehensive testing results for LNP size and the associated statistical analysis is shown in Table 3.2.P.5.6-10.

Table 3.2.P.5.6-10. BNT162b2 Drug Product Release Data for LNP Size: Statistical Analysis

Description	Clinical	Commercial / Emergency Use
N (# of lots):	[redacted]	
Mean:		
SD:		
Mean – 3SD:		
Mean + 3SD:		
Min, Max:		
[redacted]		

[2] Akinc A, Querbes W, De S, et al. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. Mol Ther 2010; 18(7): 1357-64.

[3] Rejman, J, Oberle V, Zuhorn, IS et al. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem J 2004; 377(1): 159-69.

The acceptance criterion at release and on stability for the commercial / emergency supply material has been [redacted] Because the data generated to date for the LNP size for commercial / emergency supply has all been within a narrower range as shown, the proposed release specification will be tightened to [redacted]. Understanding there is no observed increase in LNP size at the recommended storage while also recognizing the need to enable some limited storage at accelerated temperatures of -20 °C (2 weeks for distribution, 2 weeks for point of use) and 5 °C (1 month for point of use), the introduction of a "stability" acceptance criterion of 50-107 nm is proposed.

Recently (following the acquisition of all lot data used for the acceptance criteria statistical assessment), the viscosity value used for calculation of all LNP size measurements was amended, based on experimental measurement, to reflect more accurately the diluent (phosphate buffered saline) used during measurement. The corrected viscosity was determined to be 0.91 cP at 25 °C, whereas the previously used value was 1.02 cP at 25 °C.

Future measurements of LNP size will reflect this change. Consequently, a change in the acceptance criteria proposed for release and stability must be implemented as well. All data contained in Table 3.2.P.5.6-34 have been corrected/recalculated by multiplying by the 1.02 cP/0.91 cP ratio, in order to

express the results in terms of the experimentally determined viscosity. The corrected data are found in an adjacent column in Table 3.2.P.5.6-34. A corresponding correction has been incorporated into the acceptance criterion for this assay as shown in Table 3.2.P.5.6-11.

Table 3.2.P.5.6-11. Commercial Release and Stability Acceptance Criterion for LNP Size

Quality Attribute	Acceptance Criteria	Acceptance Criteria, corrected[a]
LNP Size	[redacted] (Release)	[redacted] (Release)
	[redacted] (Stability)	[redacted] (Stability)

a. Acceptance criteria corrected for amendment of viscosity value

LNP Polydispersity Index (PDI)

LNP PDI is a measure of BNT162b2 drug product homogeneity and an indication of manufacturing process control. LNP PDI is also correlated to stability. The PDI provides a measure of the distribution of size populations (i.e. polydispersity) and its numerical value ranges from [redacted] Manufacturing experience to date for BNT162b2 drug product has produced drug product values for LNP PDI that are between [redacted] (Table 3.2.P.5.6-34). Several publications suggest that a PDI value of 0.3 and below indicates a homogeneous size population for LNP[4, 5, 6].

Because the LNP PDI data are reported to only one significant digit, the data set based on the reportable result had insufficient precision to verify normal distribution of the data for statistical evaluation. As a result, the raw data for release were mined for additional precision and subsequently assessed statistically. (See Table 3.2.P.5.6-34 for PDI release data and these data expressed with an additional significant digit). The statistical assessment of the data to determine a one-sided limit (mean + 3SD) leads to a calculated upper limit of [redacted] for the commercial / emergency supply lots (n=83) and of [redacted] for the Clinical lots (n=10).

A summary of the comprehensive testing results for LNP PDI and the associated statistical analysis is shown in Table 3.2.P.5.6-12. As summarized in Section 3.2.P.8.1 Stability Summary and Conclusion, no apparent trend in LNP PDI data has been observed up through 6 months at recommended storage (-90 to -60 °C) for commercial / emergency supply and through 9 months at recommended storage (-70 °C) for supporting lot BCV40420-A, manufactured with process 1 drug substance. (See Section 3.2.P.8.3 Long-Term).

Table 3.2.P.5.6-12. BNT162 b2 Drug Product Release Data for LNP Polydispersity (PDI)[a]: Statistical Analysis

Description	Clinical	Commercial / Emergency Use
	Lots in Clinic	*All Commercial Scale Lots*
N (# of lots):	[redacted]	
Mean:		
Standard Deviation:		
Mean + 3SD:		
Min, Max:		

a. All raw data used in analysis were expressed to 2 significant digits. Release data are reported to 1 significant digit.

[4] Badran M. Formulation and in vitro evaluation of flufenamic acid loaded deformable liposome for improved skin delivery. Digest J Nanomater Biostruct 2014; 9:83-91.

[5] Chen M, Liu X, Fahr A Skin penetration and deposition of carboxyfluorescein and temoporfin from different lipid vesicular systems: In vitro study with finite and infinite dosage application. Int J Pharm 2011; 408:223-34.

[6] Putri DC, Dwiastuti R, Marchaban M, Nugroho AK Optimization of mixing temperature and sonication duration in liposome preparation. J Pharm Sci Commun 2017; 14:79-85.

Although there is no apparent trend in stability observed at the recommended storage condition, when evaluating the stability data for potential trending at the accelerated conditions of -20 °C and 5 °C durin 1 month storage (the allowance associated with the point of use), the presence of a trend is difficult to definitely determine, both because of the limited data set (≤ 4 data points) within the 1 month duration, and also due to the known method variability which was demonstrated to be approximately [redacted] as determined for intermediate precision during the method validation studies. Figure 3.2.P.5.6-1 contains BNT162b2 drug product PDI stability data through 3 months storage at -90 °C to -60 °C (recommended condition, -70 °C).

Figure 3.2.P.5.6-1. PDI Stability Data for BNT162b2: -70 °C for 3 Months

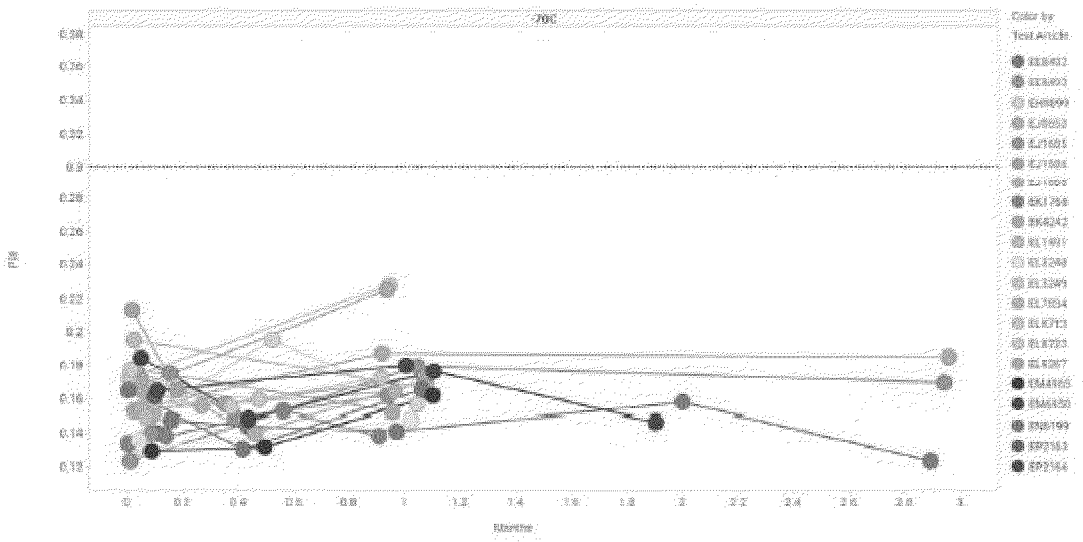


Figure 3.2.P.5.6-2 and Figure 3.2.P.5.6-3 show the plots of the BNT162b2 drug product PDI stability data through 1 month (expressed to two significant digits) for samples stored at -20 °C and 5 °C. Although visually, it may appear that there is an increase in PDI on stability across 1 month storage at the accelerated storage conditions (-20 °C and 5 °C), for many lots shown there are limited data points plotted and the apparent increase is within 1-2 calculated standard deviations of the time zero result, making it difficult to discern a true trend from analytical variability. In the case of the PDI data for BNT162b2 stored at the recommended storage condition, while there are still limited lots that have data at up to 3 months, there does not appear to be any significant trend, which is consistent with the stability analysis shown in Section 3.2.P.8.3 Long Term using data expressed to a single significant digit.

Figure 3.2.P.5.6-2. PDI Stability Data for BNT162b2: -20 °C for 1 Month

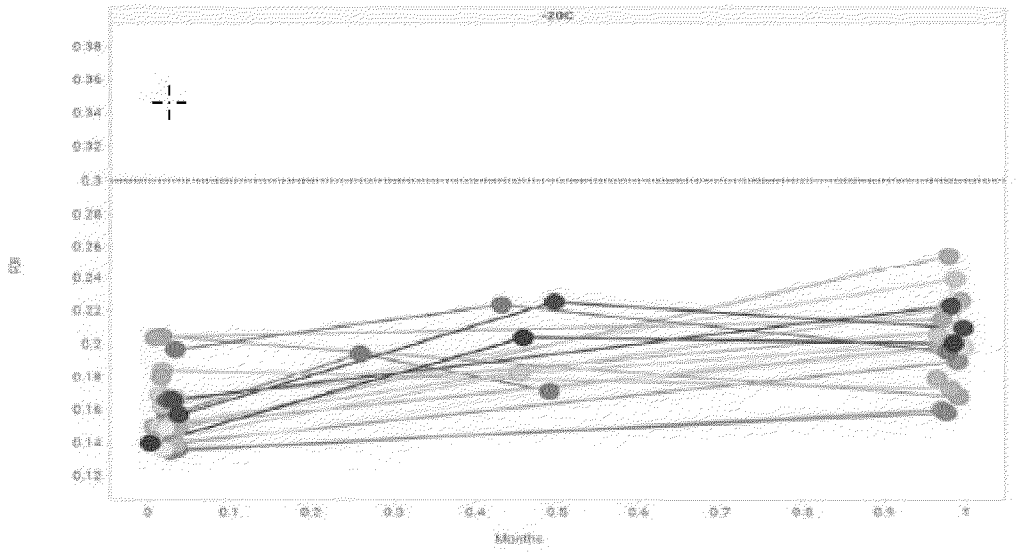
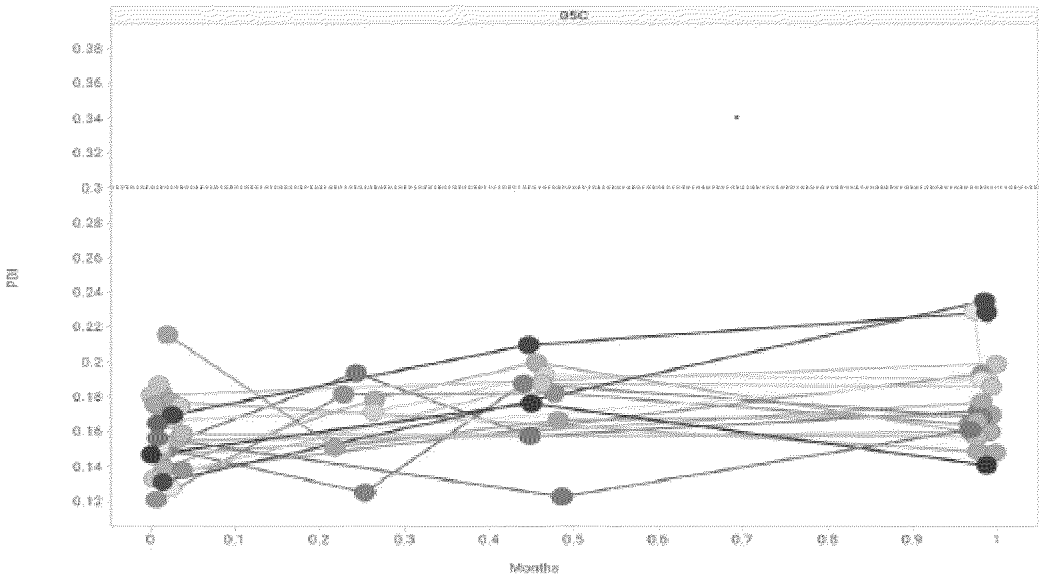


Figure 3.2.P.5.6-3. PDI Stability Data for BNT162b2: 5 °C for 1 Month



The stability data were subsequently assessed by performing variance component analysis (VCA), assuming no stability trend, therefore the variation between the time points within the same lot is considered as analytical variability. The calculated standard deviation determined from VCA accounts for the manufacturing and analytical variability. For this assessment, all stability data (expressed to two decimal places) for each lot at a specific temperature condition was included. These data can be found in Table 3.2.P.5.6-37 and Table 3.2.P.5.6-38, respectively for the -20 °C and 5 °C data sets. A mean value for the PDI attribute for each lot at each temperature (-20 °C and 5 °C) was determined by averaging together all stability values and the release value, from 83 drug product lots, and an overall mean value was determined at each temperature for PDI. The overall mean determined for the combined stability and release results at each accelerated storage condition is shown in Table 3.2.P.5.6-13 as 0.17 for the -20 °C and 0.16 for the 5 °C assessment.

The calculated standard deviations determined by variance component analysis of the two data sets based on the accelerated stability data for the two conditions (-20 °C and 5 °C) are shown in Table 3.2.P.5.6-13. For the -20 °C data set, the standard deviation calculated from the VCA was [redacted]

whereas for the 5 °C data set it was [redacted] (see Table 3.2.P.5.6-13). The standard deviation determined using the 5 °C data set was very similar to the standard deviation calculated from the release data only (standard deviation of [redacted], and reproduced for comparison in Table 3.2.P.5.6-13.

Table 3.2.P.5.6-13. BNT162 b2 Drug Product (PDI)[a]: Statistical Analysis Using Variance Component Analysis (VCA)

Description	Commercial / Emergency Supply (Release Only)	Release + Stability (-20 °C up to 1 M)	Release + Stability (5 °C up to 1 M)
N (# of lots):		[redacted]	
Mean:			
Standard Deviation:			
Mean + 3SD:			
Min, Max:			

a. All raw data used in analysis were expressed to 2 significant digits. Release data are reported to 1 significant digit.
b. Standard Deviation determined from variance component analysis (VCA)

Calculating an upper limit using the (-20 °C) VCA derived standard deviation of 0.033 gives a value of 0.27 for PDI. We note that the maximum value for PDI in the -20 °C data set is [redacted]. Both of these values round to [redacted], when expressed to the same precision as the current specification.

The proposed commercial acceptance criterion for release and on stability is presented in Table 3.2.P.5.6-14. The [redacted] limit for PDI is supported by the demonstrated assay variability reflected in PDI values for commercial / emergency use and clinical supply at release and on stability for up to 1 month at -20 °C that are at or within expected analytical variability of the current [redacted] limit. The standard deviation (0.033) determined from the variance component analysis leads to a calculated upper limit for the [redacted] of [redacted], as well.

The commercial acceptance criterion at release and on stability is presented in Table 3.2.P.5.6-14.

Table 3.2.P.5.6-14. Commercial Release and Stability Acceptance Criterion for LNP Polydispersity

Quality Attribute	Acceptance Criterion
LNP Polydispersity	[redacted]

BNT162b2 Drug Product Release Data Summary

Table 3.2.P.5.6-34 and Table 3.2.P.5.6-35 contain the drug product release testing data for one nonclinical lot, four clinical lots with associated fill/finish sublots, and 83 commercial/emergency use lots (including process performance qualification lots for several manufacturing nodes). These data have been used for the statistical assessment performed in conjunction with setting acceptance criteria for drug product quality attributes.

Table 3.2.P.5.6-34. Drug Product Release Data Summary[a] - Set 1

Lot #	DOM	Appearance (Visible Particulates)[b]	Subvisible Particles		pH	Osmolality (mOsmol/kg)	Dynamic Light Scattering				Fluorescence Assay	
			Particles ≥ 25 µm	Particles ≥ 10 µm			LNP Size (nm)	LNP Size (nm), corrected	LNP PDI	LNP PDI (2 decimal places)	RNA Encapsulation (%)	RNA Content (mg/mL)
CoVVAC270320	27-Mar-2020	NT										
BCV40420-A	30-Apr-2020	F										
BCV40620-A	24-Jun-2020	F										
BCV40620-B	25-Jun-2020	F										
BCV40620-C	26-Jun-2020	F										
BCV40620-D	29-Jun-2020	F										
BCV40620-E	30-Jun-2020	F										
BCV40720-A	23-Jul-2020	F										
BCV40720-B	24-Jul-2020	F										
BCV40720-C	25-Jul-2020	F										
BCV40720-P (ED3938)	16-Jul-2020	F										
BCV40820-P (EE3813)	29-Jul-2020	F										
EE8492	05-Aug-2020	E[c]										
EE8493	05-Aug-2020	E										
EJ0553	25-Sep-2020	E										
EJ0724	29-Sep-2020	E										
EJ1685	05-Oct-2020	E										
EJ1686	07-Oct-2020	E										
EH9899	08-Oct-2020	E										
EJ1688	12-Oct-2020	E										
EK4175	12-Oct-2020	E										
EK4176	16-Oct-2020	E										
EK1768	16-Oct-2020	E										
EK5730	23-Oct-2020	E										
EL0140	29-Oct-2020	E										
EL0141	29-Oct-2020	E										
EL0142	29-Oct-2020	E										
EL0725	30-Oct-2020	E										
EL0739	03-Nov-2020	E										
EL1484	04-Nov-2020	E										
EK9231	04-Nov-2020	E										
EK4237	05-Nov-2020	M, E										
EK4243	05-Nov-2020	M, E										
EK4244	05-Nov-2020	M, E										
EL1283	11-Nov-2020	M, E										
EJ6795	12-Nov-2020	M, E										

Table 3.2.P.5.6-34. Drug Product Release Data Summary[a] - Set 1

Lot #	DOM	Appearance (Visible Particulates)[b]	Subvisible Particles		pH	Osmolality (mOsmol/kg)	Dynamic Light Scattering				Fluorescence Assay	
			Particles ≥ 25 µm	Particles ≥ 10 µm			LNP Size (nm)	LNP Size (nm), corrected	LNP PDI	LNP PDI (2 decimal places)	RNA Encapsulation (%)	RNA Content (mg/mL)
EK4241	12-Nov-2020	M, E										
EK4245	12-Nov-2020	M, E										
EJ6796	13-Nov-2020	M, E										
EJ6797	17-Nov-2020	M, E										
EK4238	17-Nov-2020	M, E										
EK4240	17-Nov-2020	M, E										
EK4242	17-Nov-2020	M, E										
EL1284	17-Nov-2020	M, E										
EL7834	17-Nov-2020	M, E										
EL1491	18-Nov-2020	M, E										
EL3246	20-Nov-2020	M, E										
EJ3002	24-Nov-2020	M, E										
EL0200	24-Nov-2020	M, E										
EL0203	24-Nov-2020	M, E										
EM0477	24-Nov-2020	M, E										
EL3248	25-Nov-2020	M, E										
EJ6134	26-Nov-2020	M, E										
EJ6136	27-Nov-2020	M, E										
EJ6788	30-Nov-2020	M, E										
EL1404	01-Dec-2020	M, E										
EL3249	02-Dec-2020	M, E										
EK9788	03-Dec-2020	M, E										
EL1406	03-Dec-2020	M, E										
EN3924	03-Dec-2020	M, E										
EL3247	05-Dec-2020	M, E										
EJ6789	07-Dec-2020	M, E										
EL3302	07-Dec-2020	M, E										
EL8982	09-Dec-2020	M, E										
EJ6790	10-Dec-2020	M, E										
EL8723	11-Dec-2020	M, E										
EM6950	11-Dec-2020	M, E										
EL9261	12-Dec-2020	M, E										
EL9262	15-Dec-2020	M, E										
EN1185	16-Dec-2020	M, E										
EL9263	17-Dec-2020	M, E										
EN9581	17-Dec-2020	M, E										
EN5318	19-Dec-2020	M, E										
EL9266	21-Dec-2020	M, E										

Table 3.2.P.5.6-34. Drug Product Release Data Summary[a] - Set 1

Lot #	DOM	Appearance (Visible Particulates)[b]	Subvisible Particles		pH	Osmolality (mOsmol/kg)	Dynamic Light Scattering				Fluorescence Assay	
			Particles ≥ 25 µm	Particles ≥ 10 µm			LNP Size (nm)	LNP Size (nm), corrected	LNP PDI	LNP PDI (2 decimal places)	RNA Encapsulation (%)	RNA Content (mg/mL)
EL9265	22-Dec-2020	M, E	[redacted]									
EL8713	23-Dec-2020	M, E										
EP2163	23-Dec-2020	MT[d]										
EP2166	23-Dec-2020	MT										
EL9267	29-Dec-2020	M, E										
EL9269	30-Dec-2020	M, E										
EL9264	31-Dec-2020	M, E										
EM9809	01-Jan-2021	M, E										
EM9810	04-Jan-2021	M, E										
EP6775	04-Jan-2021	M, E										
EN6200	05-Jan-2021	M, E										
EN6201	07-Jan-2021	M, E										
EN1195	08-Jan-2021	M, E										
EP6017	11-Jan-2021	M, E										
EP9598	12-Jan-2021	MT										
EN6198	13-Jan-2021	M, E										
EP9605	13-Jan-2021	MT										
EN1196	18-Jan-2021	M, E										
EN6199	19-Jan-2021	M, E										
EM4965	20-Jan-2021	M, E										
ET0384	28-Jan-2021	M, E										

a. Appearance data for all lots = "White to off-white suspension";
b. Appearance (visible particulates) result abbreviations: F= Free from observable particles; E= Essentially free from visible particulates; M = Meets; MT = Meets test;
c. Change in reporting corresponds to change in acceptance criterion.
d. Difference in reporting occurred due to changes in testing location.
Other abbreviation: NT= Not tested;

Table 3.2.P.5.6-35. Drug Product Release Data Summary - Set 2

Lot #	DOM	HPLC-CAD (Lipid Content)				In Vitro Expression	Capillary Gel Electrophoresis	
		ALC-0315 (mg/mL)	ALC-0159 (mg/mL)	DSPC (mg/mL)	Cholesterol (mg/mL)	(%)	RNA Integrity (%)	LMS (%)
CoVVAC270320	27-Mar-2020	[redacted]						
BCV40420-A	30-Apr-2020							
BCV40620-A	24-Jun-2020							
BCV40620-B	25-Jun-2020							
BCV40620-C	26-Jun-2020							
BCV40620-D	29-Jun-2020							
BCV40620-E	30-Jun-2020							
BCV40720-A	23-Jul-2020							
BCV40720-B	24-Jul-2020							
BCV40720-C	25-Jul-2020							
BCV40720-P (ED3938)	16-Jul-2020							
BCV40820-P (EE3813)	29-Jul-2020							
EE8492	05-Aug-2020							
EE8493	05-Aug-2020							
EJ0553	25-Sep-2020							
EJ0724	29-Sep-2020							
EJ1685	05-Oct-2020							
EJ1686	07-Oct-2020							
EH9899	08-Oct-2020							
EJ1688	12-Oct-2020							
EK4175	12-Oct-2020							
EK4176	16-Oct-2020							
EK1768	16-Oct-2020							
EK5730	23-Oct-2020							
EL0140	29-Oct-2020							
EL0141	29-Oct-2020							
EL0142	29-Oct-2020							
EL0725	30-Oct-2020							
EL0739	03-Nov-2020							
EL1484	04-Nov-2020							
EK9231	04-Nov-2020							
EK4237	05-Nov-2020							
EK4243	05-Nov-2020							
EK4244	05-Nov-2020							
EL1283	11-Nov-2020							
EJ6795	12-Nov-2020							
EK4241	12-Nov-2020							
EK4245	12-Nov-2020							

Table 3.2.P.5.6-35. Drug Product Release Data Summary - Set 2

Lot #	DOM	HPLC-CAD (Lipid Content)				In Vitro Expression	Capillary Gel Electrophoresis	
		ALC-0315 (mg/mL)	ALC-0159 (mg/mL)	DSPC (mg/mL)	Cholesterol (mg/mL)	(%)	RNA Integrity (%)	LMS (%)
EJ6796	13-Nov-2020	[redacted]						
EJ6797	17-Nov-2020							
EK4238	17-Nov-2020							
EK4240	17-Nov-2020							
EK4242	17-Nov-2020							
EL1284	17-Nov-2020							
EL7834	17-Nov-2020							
EL1491	18-Nov-2020							
EL3246	20-Nov-2020							
EJ3002	24-Nov-2020							
EL0200	24-Nov-2020							
EL0203	24-Nov-2020							
EM0477	24-Nov-2020							
EL3248	25-Nov-2020							
EJ6134	26-Nov-2020							
EJ6136	27-Nov-2020							
EJ6788	30-Nov-2020							
EL1404	01-Dec-2020							
EL3249	02-Dec-2020							
EK9788	03-Dec-2020							
EL1406	03-Dec-2020							
EN3924	03-Dec-2020							
EL3247	05-Dec-2020							
EJ6789	07-Dec-2020							
EL3302	07-Dec-2020							
EL8982	09-Dec-2020							
EJ6790	10-Dec-2020							
EL8723	11-Dec-2020							
EM6950	11-Dec-2020							
EL9261	12-Dec-2020							
EL9262	15-Dec-2020							
EN1185	16-Dec-2020							
EL9263	17-Dec-2020							
EN9581	17-Dec-2020							
EN5318	19-Dec-2020							
EL9266	21-Dec-2020							
EL9265	22-Dec-2020							
EL8713	23-Dec-2020							
EP2163	23-Dec-2020							
EP2166	23-Dec-2020							

Table 3.2.P.5.6-35. Drug Product Release Data Summary - Set 2

Lot #	DOM	HPLC-CAD (Lipid Content)				In Vitro Expression	Capillary Gel Electrophoresis	
		ALC-0315 (mg/mL)	ALC-0159 (mg/mL)	DSPC (mg/mL)	Cholesterol (mg/mL)	(%)	RNA Integrity (%)	LMS (%)
EL9267	29-Dec-2020	[redacted]						
EL9269	30-Dec-2020							
EL9264	31-Dec-2020							
EM9809	01-Jan-2021							
EM9810	04-Jan-2021							
EP6775	04-Jan-2021							
EN6200	05-Jan-2021							
EN6201	07-Jan-2021							
EN1195	08-Jan-2021							
EP6017	11-Jan-2021							
EP9598	12-Jan-2021							
EN6198	13-Jan-2021							
EP9605	13-Jan-2021							
EN1196	18-Jan-2021							
EN6199	19-Jan-2021							
EM4965	20-Jan-2021							
ET0384	28-Jan-2021							

NT= Not tested; ND = Not detected

BNT162b2 Drug Product PDI Stability Data

Drug product data for PDI (expressed to 2 decimal places) from lots stored at -70 °C (recommended), -20 °C and 5 °C (accelerated) are contained in Table 3.2.P.5.6-36, Table 3.2.P.5.6-37, and Table 3.2.P.5.6-38.

Table 3.2.P.5.6-36. BNT162b2 Drug Product Stability Data at -70 °C for PDI

Lot	Months	Condition	Level	PDI (1 decimal)	PDI (2 decimals)
EE8492	0	-70C	UPRIGHT	[redacted]	
EE8492	0.23	-70C	UPRIGHT		
EE8492	0.46	-70C	UPRIGHT		
EE8492	1	-70C	UPRIGHT		
EE8492	2	-70C	UPRIGHT		
EE8492	3	-70C	UPRIGHT		
EE8493	0	-70C	UPRIGHT		
EE8493	1	-70C	UPRIGHT		
EE8493	3	-70C	UPRIGHT		
EJ0553	0	-70C	UPRIGHT		
EJ0553	1	-70C	UPRIGHT		
EJ0553	3	-70C	UPRIGHT		
EJ1685	0	-70C	UPRIGHT		
EJ1685	0.23	-70C	UPRIGHT		
EJ1685	0.46	-70C	UPRIGHT		
EJ1685	1	-70C	UPRIGHT		
EJ1688	0	-70C	UPRIGHT		
EJ1688	0.23	-70C	UPRIGHT		
EJ1688	0.46	-70C	UPRIGHT		
EJ1688	1	-70C	UPRIGHT		
EH9899	0	-70C	UPRIGHT		
EH9899	0.23	-70C	UPRIGHT		
EH9899	0.46	-70C	UPRIGHT		
EH9899	1	-70C	UPRIGHT		
EJ1686	0	-70C	UPRIGHT		
EJ1686	0.23	-70C	UPRIGHT		
EJ1686	0.46	-70C	UPRIGHT		
EJ1686	1	-70C	UPRIGHT		
EK1768	0	-70C	UPRIGHT		
EK1768	0.23	-70C	UPRIGHT		
EK1768	0.46	-70C	UPRIGHT		
EK1768	1	-70C	UPRIGHT		
EL7834	0	-70C	INVERTED		
EL7834	0	-70C	UPRIGHT		
EL7834	1	-70C	UPRIGHT		
EK4242	0	-70C	INVERTED		
EK4242	0	-70C	UPRIGHT		
EK4242	1	-70C	UPRIGHT		
EL1491	0	-70C	INVERTED		
EL1491	0	-70C	UPRIGHT		
EL1491	1	-70C	UPRIGHT		
EL3248	0	-70C	INVERTED		
EL3248	0	-70C	UPRIGHT		

Table 3.2.P.5.6-36. BNT162b2 Drug Product Stability Data at -70 °C for PDI

Lot	Months	Condition	Level	PDI (1 decimal)	PDI (2 decimals)
EL3248	1	-70C	UPRIGHT	[redacted]	
EL3249	0	-70C	INVERTED		
EL3249	0	-70C	UPRIGHT		
EL3249	1	-70C	UPRIGHT		
EL8723	0	-70C	UPRIGHT		
EL8723	0.46	-70C	UPRIGHT		
EM6950	0	-70C	UPRIGHT		
EM6950	0.46	-70C	UPRIGHT		
EM6950	1	-70C	UPRIGHT		
EM6950	2	-70C	UPRIGHT		
EL8713	0	-70C	UPRIGHT		
EL8713	0.46	-70C	UPRIGHT		
EL8713	1	-70C	UPRIGHT		
EP2163	0	-70C	UPRIGHT		
EP2166	0	-70C	UPRIGHT		
EP2166	1	-70C	UPRIGHT		
EL9267	0	-70C	UPRIGHT		
EL9267	1	-70C	UPRIGHT		
EN6199	0	-70C	UPRIGHT		
EN6199	0.46	-70C	UPRIGHT		
EN6199	1	-70C	UPRIGHT		
EM4965	0	-70C	UPRIGHT		
EM4965	0.46	-70C	UPRIGHT		
EM4965	1	-70C	UPRIGHT		

Table 3.2.P.5.6-37. BNT162b2 Drug Product Stability Data -20 °C for PDI

Lot	Months	Condition	Level	PDI (1 decimal)	PDI (2 decimals)
EE8492	0	-20C	UPRIGHT		
EE8492	0.23	-20C	UPRIGHT		
EE8492	0.46	-20C	UPRIGHT		
EE8492	1	-20C	UPRIGHT		
EE8493	0	-20C	UPRIGHT		
EE8493	1	-20C	UPRIGHT		
EJ0553	0	-20C	UPRIGHT		
EJ0553	1	-20C	UPRIGHT		
EJ1685	0	-20C	UPRIGHT		
EJ1685	1	-20C	UPRIGHT		
EJ1688	0	-20C	UPRIGHT		
EJ1688	1	-20C	UPRIGHT		
EH9899	0	-20C	UPRIGHT		
EH9899	1	-20C	UPRIGHT		
EJ1686	0	-20C	UPRIGHT		
EJ1686	1	-20C	UPRIGHT		
EK1768	0	-20C	UPRIGHT		
EK1768	1	-20C	UPRIGHT		
EL7834	0	-20C	INVERTED		
EL7834	1	-20C	INVERTED		
EL7834	0	-20C	UPRIGHT		
EL7834	1	-20C	UPRIGHT		
EK4242	0	-20C	INVERTED		
EK4242	1	-20C	INVERTED		
EK4242	0	-20C	UPRIGHT		
EK4242	1	-20C	UPRIGHT		
EL1491	0	-20C	INVERTED		
EL1491	0	-20C	UPRIGHT		
EL1491	1	-20C	UPRIGHT		
EL3248	0	-20C	INVERTED		
EL3248	1	-20C	INVERTED		
EL3248	0	-20C	UPRIGHT		
EL3248	1	-20C	UPRIGHT		
EL3249	0	-20C	INVERTED		
EL3249	1	-20C	INVERTED		
EL3249	0	-20C	UPRIGHT		
EL3249	1	-20C	UPRIGHT		
EL8723	0	-20C	UPRIGHT		
EM6950	0	-20C	UPRIGHT		
EM6950	0.46	-20C	UPRIGHT		
EM6950	1	-20C	UPRIGHT		
EL8713	0	-20C	UPRIGHT		
EL8713	0.46	-20C	UPRIGHT		
EL8713	1	-20C	UPRIGHT		
EP2163	0	-20C	UPRIGHT		
EP2166	0	-20C	UPRIGHT		
EP2166	1	-20C	UPRIGHT		
EL9267	0	-20C	UPRIGHT		
EL9267	1	-20C	UPRIGHT		

Table 3.2.P.5.6-37. BNT162b2 Drug Product Stability Data -20 °C for PDI

Lot	Months	Condition	Level	PDI (1 decimal)	PDI (2 decimals)
EN6199	0	-20C	UPRIGHT	[redacted]	
EN6199	0.46	-20C	UPRIGHT		
EN6199	1	-20C	UPRIGHT		
EM4965	0	-20C	UPRIGHT		
EM4965	0.46	-20C	UPRIGHT		
EM4965	1	-20C	UPRIGHT		

Table 3.2.P.5.6-38. BNT162b2 Drug Product Stability Data at 5 °C for PDI

Lot	Months	Condition	Level	PDI (1 decimal)	PDI (2 decimals)
EE8492	0	05C	UPRIGHT	[redacted]	
EE8492	0.23	05C	UPRIGHT		
EE8492	0.46	05C	UPRIGHT		
EE8492	1	05C	UPRIGHT		
EE8493	0	05C	UPRIGHT		
EE8493	1	05C	UPRIGHT		
EJ0553	0	05C	UPRIGHT		
EJ0553	1	05C	UPRIGHT		
EJ1685	0	05C	UPRIGHT		
EJ1685	0.23	05C	UPRIGHT		
EJ1685	0.46	05C	UPRIGHT		
EJ1685	1	05C	UPRIGHT		
EJ1688	0	05C	UPRIGHT		
EJ1688	0.23	05C	UPRIGHT		
EJ1688	0.46	05C	UPRIGHT		
EJ1688	1	05C	UPRIGHT		
EH9899	0	05C	UPRIGHT		
EH9899	0.23	05C	UPRIGHT		
EH9899	0.46	05C	UPRIGHT		
EH9899	1	05C	UPRIGHT		
EJ1686	0	05C	UPRIGHT		
EJ1686	0.23	05C	UPRIGHT		
EJ1686	0.46	05C	UPRIGHT		
EJ1686	1	05C	UPRIGHT		
EK1768	0	05C	UPRIGHT		
EK1768	0.23	05C	UPRIGHT		
EK1768	0.46	05C	UPRIGHT		
EK1768	1	05C	UPRIGHT		
EL7834	0	05C	INVERTED		
EL7834	1	05C	INVERTED		
EL7834	0	05C	UPRIGHT		
EL7834	1	05C	UPRIGHT		
EK4242	0	05C	INVERTED		
EK4242	1	05C	INVERTED		
EK4242	0	05C	UPRIGHT		
EK4242	1	05C	UPRIGHT		
EL1491	0	05C	INVERTED		
EL1491	0	05C	UPRIGHT		
EL1491	1	05C	UPRIGHT		
EL3248	0	05C	INVERTED		
EL3248	1	05C	INVERTED		
EL3248	0	05C	UPRIGHT		
EL3248	1	05C	UPRIGHT		
EL3249	0	05C	INVERTED		
EL3249	1	05C	INVERTED		
EL3249	0	05C	UPRIGHT		
EL8723	0	05C	UPRIGHT		
EM6950	0	05C	UPRIGHT		
EM6950	0.46	05C	UPRIGHT		

Table 3.2.P.5.6-38. BNT162b2 Drug Product Stability Data at 5 °C for PDI

Lot	Months	Condition	Level	PDI (1 decimal)	PDI (2 decimals)
EM6950	1	05C	UPRIGHT	[redacted]	
EL8713	0	05C	UPRIGHT		
EL8713	0.46	05C	UPRIGHT		
EL8713	1	05C	UPRIGHT		
EP2163	0	05C	UPRIGHT		
EP2166	0	05C	UPRIGHT		
EP2166	1	05C	UPRIGHT		
EL9267	0	05C	UPRIGHT		
EN6199	0	05C	UPRIGHT		
EN6199	0.46	05C	UPRIGHT		
EN6199	1	05C	UPRIGHT		
EM4965	0	05C	UPRIGHT		
EM4965	0.46	05C	UPRIGHT		
EM4965	1	05C	UPRIGHT		

RNA Encapsulation

RNA encapsulation in BNT162b2 drug product is measured by a fluorescence assay in which free RNA and total RNA (measurable following release of encapsulated RNA from the lipid nanoparticles (LNP)) are measured relative to a standard curve. The difference between the total RNA and the free RNA corresponds to the relative RNA encapsulation. The efficacy of the BNT162b2 drug product is dependent on LNP encapsulation of the RNA drug substance.

Encapsulation is used to ensure delivery of the RNA and improve the chances of transfection.

Originally developed for entrapment of DNA plasmids, Bailey and Sullivan[7] demonstrated that entrapment of up to 80% of DNA plasmids inside stable, uncharged liposomes smaller than 200 nm on average provided a two- to three-fold improvement over the best trapping efficiency previously reported for liposomes. Subsequently Jeffs et al.[8] produced liposomal encapsulated plasmid DNA using lipids dissolved in ethanol mixed with an aqueous DNA solution, making monodisperse vesicles with particle sizes less than 200 nm and DNA encapsulation efficiencies greater than 80%, which demonstrated good tumor accumulation and gene expression. An additional report by Hassett et al.[9], in which LNPs were used to encapsulate mRNA at an efficiency of >72%, demonstrated a robust immune response. These studies suggest that encapsulation at or above [redacted] is an appropriate acceptance criterion.

During formulation development (Section 3.2.P.2.2 Drug Product), the impact of the citrate buffer strength (RNA diluted in citrate, prior to mixing with lipids), the ALC-0315 to RNA (N/P) molar ratio and the mixing rate for the encapsulation step were all evaluated and the results demonstrated that RNA encapsulation [redacted] was achievable. Encapsulation levels at or above [redacted] have been consistently demonstrated for drug product lots throughout development and commercial / emergency supply manufacture (Table 3.2.P.5.6-34). A summary of the comprehensive testing results and the associated statistical analysis is shown in Table 3.2.P.5.6-15.

[7] Bailey AL, Sullivan SM Efficient Encapsulation of DNA Plasmids in Small Neutral Liposomes Induced by Ethanol and Calcium. Biochim Biophys Acta 2000;1468(1-2):239-52.

[8] Jeffs LB, Palmer LR, Ambegia EG, et al. A Scalable, Extrusion-Free Method for Efficient Liposomal Encapsulation of Plasmid DNA. Pharm Research 2005;22(3):362-72.

[9] Hassett KJ, Benenato KE, Jacquinet E, et al. Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. Mol Ther Nucleic Acids 2019;15:1-11.

Table 3.2.P.5.6-15. BNT162b2 Drug Product Release Data for RNA Encapsulation: Statistical Analysis

Description	Clinical	Commercial /Emergency Use
	Lots in Clinic	*All Commercial Scale Lots*
N (# of lots):		
Mean:		
Standard Deviation:		
Min, Max:		
[redacted]		

The statistical assessment of the data to determine a [redacted] leads to a calculated lower limit of [redacted] for both the commercial / emergency use lots (n=83) and the clinical lots (n=10). Data from the Dajan et al. publication show that patients immunized with a lot (EK4240) containing [redacted] encapsulated RNA are included in the data set for which a robust immune response was demonstrated, verifying that RNA encapsulation at [redacted] is efficacious. No trend in RNA encapsulation data through 3 months and up through 9 months at recommended storage (Section 3.2.P.8.3 Long Term) for commercial / emergency use lots and representative clinical lots (drug product manufactured with process 1 drug substance), respectively, has been observed. A small apparent decrease in encapsulation has been observed at -20 °C for the commercial / emergency use lots across 1 M storage (Section 3.2.P.8.3 Accelerated), but no significant trend is observed across 1 M storage at 5 °C; all results are well within the proposed limits (see Section 3.2.P.8.1 Stability Summary and Conclusion).

The statistical analysis in conjunction with the recently published study reporting on vaccine efficacy in the Israeli population and literature studies supporting [redacted] encapsulation as providing a robust immune response support a proposed specification for RNA encapsulation at release and on stability of [redacted] as presented in Table 3.2.P.5.6-16. This proposed acceptance criterion represents a tightening of 5% relative to the criterion in place at time of commercial / emergency use drug product lot release.

Table 3.2.P.5.6-16. Commercial Release and Stability Acceptance Criterion for RNA Encapsulation

Quality Attribute	Acceptance Criterion
RNA Encapsulation	[redacted]

RNA Content

The RNA content of BNT162b2 drug product is determined for release and on stability by measuring the total RNA against a standard curve, following release of encapsulated RNA from the LNP. A fluorescent dye (e.g., Ribogreen) that binds to RNA following the disruption of the LNP, provides a quantitative result for the total RNA content. RNA content is measured for every lot of BNT162b2 drug product at release and during stability studies to ensure that the label claim for strength is met.

The statistical treatment of the commercial / emergency supply data set to determine a two sided acceptance criterion based on the mean ± 3SD analysis was performed.

Table 3.2.P.5.6-17 contains the statistical analysis for this assessment. All stability data results for the commercial / emergency supply lots at recommended storage were within the release result range

(Section 3.2.P.8.3 Long-Term), with no observed trend in RNA content through 6 months when stored at the recommended long-term conditions. Similarly, RNA content at the accelerated conditions of -20 °C and 5 °C show no apparent trend across 1 month storage (Section 3.2.P.8.3 Accelerated).

Table 3.2.P.5.6-17. BNT162b2 Drug Product Release Data for RNA Content Statistical Assessment

Description	Clinical	Commercial / Emergency Use
	Lots in Clinic	*All Commercial Scale Lots*
N (# of lots):		
Mean:		
Standard Deviation:		
Mean – 3SD:		
Mean + 3SD:		
Min, Max:		

The proposed commercial acceptance criterion for RNA content at release and on stability is presented in Table 3.2.P.5.6-18. The commercial / emergency supply release specification was The commercial acceptance criteria is slightly broader than the range corresponding to determined from the data set, but has been tightened from the commercial / emergency supply acceptance criterion, and reflects the capability of the process as well as the assay variability, which was determined to be relative standard deviation during intermediate precision assessment for the method (Section 3.2.P.5.3 Fluorescence Assay). The proposed commercial specification also considered the dose range data for the BNT162b2 in clinical trials10. While a 30 microgram vaccine dose was selected, all three BNT162b2 vaccine doses of 10, 20 and 30 micrograms, elicited robust immune responses as demonstrated by spike protein (S1) binding IgG levels at day 28 that were higher than a convalescent serum sample from SARs-CoV2 patients. These data support that no impact to product efficacy would be expected for RNA content within the proposed commercial acceptance criterion.

Table 3.2.P.5.6-18. Commercial Release and Stability Acceptance Criterion for RNA Content

Quality Attribute	Acceptance Criterion
RNA Content	

Lipid Content

The lipids used in the vaccine DP formulation serve various functions: DSPC and cholesterol are structural lipids, providing a stable bilayer and enabling mobility of the lipid components within the LNP structure, ALC-0315 is an ionizable cationic lipid which is important for successful delivery of RNA, ensuring the self-assembly of the LNP, the uptake of the LNP into the cells, and the escape of the RNA from the endosome, while ALC-0159 is a PEGylated lipid which inserts itself in the outer lipid bilayer of the LNP, thereby providing a steric barrier to interactions with surfaces or other LNP that could result in particle fusion during storage. The content for each lipid component is measured by HPLC with either a charged aerosol detector (CAD) or an evaporative light scattering detector (ELSD) and quantified by interpolation from a standard curve. Table 3.2.P.5.6-35 contains the lipid content release testing data obtained for representative BNT162b2 drug product lots corresponding to the "clinical" lots (n=10; lots manufactured from process 1 drug substance at smaller scale) and the commercial / emergency supply lots (n=83; lots manufactured from process 2 drug substance)

During development for clinical and commercial / emergency supply manufacturing, initial acceptance criteria for lipid content were broad, reflecting the acceptable RNA content range, formulation development data, and limited manufacturing and stability experience. The content of individual lipids is roughly proportional to the RNA content, as determined by the target input levels of each for drug

product manufacture. The development study described in Section 3.2.P.2.2.1 Formulation Development demonstrates that across a broad range of ratios of the ALC-0315 to RNA, key critical quality attributes of the LNP (LNP size, PDI and encapsulation) are attained.

Lipid content data for ALC-0315, ALC-0159, DSPC and cholesterol from the eighty-three lots of commercial / emergency supply were subjected to statistical analysis to calculate two sided acceptance ranges for each lipid. The statistical assessment for lipids was performed to determine the range for each lipid using limits corresponding to [redacted] This range is justified based on an understanding that the role of the lipids is to enable RNA function in vivo, for which each lipid has a specified role and must be present, but tight control of the absolute quantity of each of the lipids is not critical, hence the proposal of slightly wider acceptance criteria. Table 3.2.P.5.6-19 contains the results of this analysis with comparison to the same analysis performed on the clinical drug product lots (n=10). The two-sided ranges calculated from the clinical vs the commercial / emergency use data sets are similar.

Table 3.2.P.5.6-19. BNT162b2 Drug Product Lipid Content Release Data: Statistical Analysis

	ALC-0315 (mg/mL)		ALC-0159 (mg/mL)		DSPC (mg/mL)		Cholesterol (mg/mL)	
	Target content [redacted] mg/mL		Target content [redacted] mg/mL		Target content [redacted] mg/mL		Target content [redacted] mg/mL	
Description	*Clinical*	*Commercial / Emergency Use*	*Clinical*	*Commercial / Emergency Use*	*Clinical*	*Commercial / Emergency Use*	*Clinical*	*Commercial / Emergency Use*
N(# of Lots)	10	83	10	83	10	83	10	83
Mean	[redacted]							
SD								
Mean – 4SD								
Mean + 4SD								
Min, Max								

There is no significant change observed in lipid content upon storage at recommended or accelerated conditions up to 6 months storage (Section 3.2.P.8.3 Long-Term, Section 3.2.P.8.3 Accelerated). Therefore, the proposed acceptance criteria for the lipid content for ALC-0315, ALC-0159, DSPC and cholesterol are based on the statistical assessment as described above, the formulation development data, the absence of trend on stability, and on the tightened RNA content range. These criteria represent a tightening from those in place for commercial / emergency supply release (ALC-0315 range previously [redacted] mg/mL; ALC-0159 range previously [redacted]; DSPC range previously [redacted] mg/mL; Cholesterol range previously [redacted] mg/mL), ensuring consistency of commercial LNPs.

Table 3.2.P.5.6-20. Commercial Release and Stability Acceptance Criteria for Lipid Content

Quality Attribute	Acceptance Criteria
ALC-0315 Content	[redacted] mg/mL
ALC-0159 Content	[redacted] mg/mL
DSPC Content	[redacted] mg/mL
Cholesterol Content	[redacted] mg/mL

In Vitro Expression

In vitro expression is a cell-based assay in which HEK293T cells are transfected with BNT162b2 drug product and the output is measured as expression of the SARS-CoV-2 S1 antigen using a bound fluorophore which enables the detection of transfected cells by flow cytometry. The acceptance criterion for BNT162b2 drug product in vitro expression is selected to show expression of the SARS-CoV-2 S1 antigen. The absolute value for % cells positive for expression is recorded. The correlation of in vitro expression with antigen binding antibody responses and SARS-CoV-2 neutralizing responses in mice immunized with the drug product is shown in Section 3.2.P.2.2 Establishing In Vitro and In Vivo Correlation. Because this assay was implemented late in development, limited data are available for historical drug product lots. All available data from comparability measurements for drug product manufactured during development (designated "clinical") and release testing results for the lots

manufactured for commercial / emergency supply are shown in Table 3.2.P.5.6-24 and Table 3.2.P.5.6-35, respectively. The comparability data for clinical lots (n=6) demonstrated an IVE range of [redacted] and the observed range for the commercial / emergency supply lot release data (n=83) was [redacted] As this is a cell-based assay relying on in vitro transfection and subsequent confirmation of expression by antibody detection and flow cytometry, there is assay-to-assay variability. Therefore, it is not surprising that the range of results for the commercial / emergency supply across many assays is wider than the range obtained from the single comparability exercise on the clinical lots. The range observed for commercial / emergency supply is considered to have appropriately confirmed the vaccine lots expressed S1 antigen.

Table 3.2.P.5.6-24. BNT162b2 Drug Product In Vitro Expression Comparability and Release Data

Lot	% Cells Positive
BCV40420-A	[redacted] (comparability)
BCV40620-A	(comparability)
BCV40620-D	(comparability)
BCV40720-A	(comparability)
ED3938	(comparability)
EE3813	(comparability)

The in vitro expression method is a limit assay and the acceptance criterion is selected to show expression of the SARS-CoV-2 S1 antigen. The statistical treatment of the commercial / emergency supply data set to calculate a one-sided acceptance criterion was performed.

The IVE data showed significant deviation from a normal distribution as determined by both the normality test and by quantile plot assessment. This data set, however, better fits a normal distribution after transformation by squaring. As a result, the calculated acceptance criterion (lower limit) for IVE was determined by first calculating the [redacted] limit based on the squared values of the IVE results followed by taking the square root of that value. The result of the analysis yielded a lower limit for the commercial / emergency use data set of [redacted].

Table 3.2.P.5.6-25 contains the statistical analysis for this assessment. All stability data results for the commercial / emergency supply lots at recommended storage were within the release result range of [redacted] (Section 3.2.P.8.3 Long-Term) and there was no observable trend in in vitro expression for up to 6 months when stored at recommended long-term conditions. No significant trend in IVE was observed for drug product stored through 1 month at accelerated conditions (-20 °C and 5 °C).

Table 3.2.P.5.6-25. BNT162b2 Drug Product In Vitro Expression (%) Data Summary: Statistical Analysis

Description	Clinical Lots	Commercial / Emergency Use Lots
N (# of lots):	[redacted]	
Mean:		
SD:		
Min, Max:		
Calculated lower limit[a]:	ND	[redacted]

a. Calculated by squaring the IVE results, determining [redacted] and taking the square root
ND = not determined

Based on this analysis combined with the statistical assessment of the commercial / emergency supply data set summarized in Table 3.2.P.5.6-25, the proposed acceptance criteria of [redacted] S1+ cells as presented in Table 3.2.P.5.6-26 for release and stability is appropriate.

Table 3.2.P.5.6-26. Commercial Release and Stability Acceptance Criterion for In Vitro Expression

Quality Attribute	Acceptance Criterion
In Vitro Expression	[redacted] Cells Positive

RNA Integrity

Capillary gel electrophoresis is routinely used to evaluate the RNA integrity of the BNT162b2 drug product at release and during stability. The method can detect potential degradation products based on their respective migration times and data are reported as relative peak area. Three main species are observed in the RNA integrity assay by CGE: fragments, main, and those species that migrate later than the main peak. The species that migrate after the main peak are observed in drug product batches and are typically not present in the final RNA drug substance. (Description and characterization of the late migrating species is described in Section 3.2.P.2.2 Drug Product, subsection 3.2.P.2.2.3.4.1 RNA Integrity). The efficacy of the drug product is dependent on the expression of the delivered RNA, which requires a sufficiently intact RNA molecule. As such, the efficacy of the BNT162b2 drug product is ensured by setting an acceptance criterion for intact RNA (i.e., the main peak within the CGE electropherogram). It is important to note that this criterion on the main peak controls both fragments and the later migrating species (LMS) since all peaks before and after the main peak are integrated and subtracted from the total peak area; that is, any increase in fragment or late migrating species results in a concomitant lowering of the reported percent RNA integrity.

The RNA integrity specification has been evaluated and is justified by the following:

-Statistical analysis of clinical and commercial / emergency use lots

-Evaluation of RNA integrity change upon drug product storage at 2 – 8 °C

-Clinical justification for the end of shelf life RNA integrity acceptance criterion including recently published real world evidence

Integrity measurements for one nonclinical lot (CoVVAC270320), four clinical/clinical inventory lots (BCV40420, BCV40620, BCV40720 and BCV40820; with associated sublots) manufactured from Process 1 drug substance and eighty-three commercial / emergency supply drug product lots manufactured (EE8492 through ET0384) from Process 2 drug substance are shown in Table 3.2.P.5.6-27 and span a range from [redacted] RNA integrity.

To establish the acceptance criterion lower limit for RNA integrity, data from all lots manufactured from BCV40420-A through ET0384 have been considered. The comparability of these drug product materials has been evaluated, as shown in Section 3.2.P.2.3 Process Development and Characterization and drug product manufactured from process 1 and 2 drug substance batches were demonstrated to be comparable based on this assessment.

Table 3.2.P.5.6-27. Drug Product Release Data Set for RNA Integrity and Associated ALC-0315 Lipid Source

Lot #	Date of Manufacture	RNA Integrity (%)	Commercial / Emergency Supply Stability Lots[a]	Lot Subset Included in Integrity Acceptance Criterion Reassessment	ALC-0315 Lipid Source[b]
CoVVAC270320	27-Mar-2020	[redacted]	NA		Avanti
BCV40420-A	30-Apr-2020				
BCV40620-A	24-Jun-2020				
BCV40620-B	25-Jun-2020				
BCV40620-C	26-Jun-2020				
BCV40620-D	29-Jun-2020				
BCV40620-E	30-Jun-2020				
BCV40720-A	23-Jul-2020				
BCV40720-B	24-Jul-2020				
BCV40720-C	25-Jul-2020				
BCV40720-P (ED3938)	16-Jul-2020				
BCV40820-P (EE3813)	29-Jul-2020				
EE8492	05-Aug-2020		X		Avanti GALC0315-12
EE8493	05-Aug-2020		X		Avanti GALC0315-13
EJ0553[c]	25-Sep-2020		X	X	Avanti GALC0315-12 and GALC0315-13
EJ0724	29-Sep-2020			X	Avanti GALC0315-14
EJ1685	05-Oct-2020		X		Croda DTP/465/3
EJ1686	07-Oct-2020		X		Croda DTP/465/3
EH9899	08-Oct-2020		X		Croda DTP/465/3
EJ1688	12-Oct-2020		X		Croda 1755889
EK4175	12-Oct-2020				
EK4176	16-Oct-2020				
EK1768	16-Oct-2020		X		Croda 1755889
EK5730	23-Oct-2020				
EL0140	29-Oct-2020				
EL0141	29-Oct-2020				
EL0142	29-Oct-2020				
EL0725	30-Oct-2020				
EL0739	03-Nov-2020				
EL1484	04-Nov-2020				
EK9231	04-Nov-2020				
EK4237	05-Nov-2020				
EK4243	05-Nov-2020				
EK4244	05-Nov-2020				
EL1283	11-Nov-2020				
EJ6795	12-Nov-2020				

Table 3.2.P.5.6-27. Drug Product Release Data Set for RNA Integrity and Associated ALC-0315 Lipid Source

Lot #	Date of Manufacture	RNA Integrity (%)	Commercial / Emergency Supply Stability Lots[a]	Lot Subset Included in Integrity Acceptance Criterion Reassessment	ALC-0315 Lipid Source[b]
EK4241	12-Nov-2020	[redacted]			
EK4245	12-Nov-2020				
EJ6796	13-Nov-2020				
EJ6797	17-Nov-2020				
EK4238	17-Nov-2020				
EK4240	17-Nov-2020				
EK4242	17-Nov-2020		X		Croda 1780226
EL1284	17-Nov-2020				
EL7834	17-Nov-2020		X		Croda 1755889 and DTP-465-3
EL1491	18-Nov-2020		X		Croda 1781853
EL3246	20-Nov-2020				
EJ3002	24-Nov-2020				
EL0200	24-Nov-2020				
EL0203	24-Nov-2020				
EM0477	24-Nov-2020				
EL3248	25-Nov-2020		X		Croda 1760275
EJ6134	26-Nov-2020				
EJ6136	27-Nov-2020				
EJ6788	30-Nov-2020				
EL1404	01-Dec-2020				
EL3249	02-Dec-2020		X		Croda 1781853
EK9788	03-Dec-2020				
EL1406	03-Dec-2020				
EN3924	03-Dec-2020				
EL3247	05-Dec-2020				
EJ6789	07-Dec-2020			X	Groton 20-AP-00483
EL3302	07-Dec-2020				
EL8982	09-Dec-2020				
EJ6790	10-Dec-2020			X	Groton 20-AP-00483
EL8723	11-Dec-2020		X		Croda 1793445
EM6950	11-Dec-2020		X	X	Croda 1796283
EL9261	12-Dec-2020			X	Avanti GALC031514 and ALC0315115
EL9262	15-Dec-2020				
EN1185	16-Dec-2020				
EL9263	17-Dec-2020				
EN9581	17-Dec-2020				
EN5318	19-Dec-2020				
EL9266	21-Dec-2020			X	Croda 1796283
EL9265	22-Dec-2020			X	Groton 20AP00489
EL8713	23-Dec-2020		X	X	Groton 20-AP-00489
EP2163	23-Dec-2020			X	Croda 1797990
EP2166	23-Dec-2020		X	X	Croda 1799380
EL9267	29-Dec-2020		X	X	Croda 1797990
EL9269	30-Dec-2020			X	Groton 20AP00489
EL9264	31-Dec-2020			X	Groton 20AP00489
EM9809	01-Jan-2021			X	Groton 20AP00489
EM9810	04-Jan-2021			X	Groton 20AP00469
EP6775	04-Jan-2021			X	Groton 20AP00495
EN6200	05-Jan-2021			X	Croda 1797990
EN6201	07-Jan-2021			X	Groton 20AP00495
EN1195	08-Jan-2021		X	X	Croda 1803068
EP6017	11-Jan-2021			X	Croda 0001803068
EP9598	12-Jan-2021			X	Groton 20-AP-00489 and 20-AP-00495
EN6198	13-Jan-2021			X	Croda 1799380
EP9605	13-Jan-2021			X	Croda 0001806330 0001803068

Table 3.2.P.5.6-27. Drug Product Release Data Set for RNA Integrity and Associated ALC-0315 Lipid Source

Lot #	Date of Manufacture	RNA Integrity (%)	Commercial / Emergency Supply Stability Lots[a]	Lot Subset Included in Integrity Acceptance Criterion Reassessment	ALC-0315 Lipid Source[b]
EN1196	18-Jan-2021	[redacted]		X	Croda 1803068
EN6199	19-Jan-2021		X	X	Croda 1803068
EM4965	20-Jan-2021		X	X	Croda 1799380
ET0384	28-Jan-2021			X	Croda 1799380

a. Shading in this column designates nonclinical and clinical lots, as well as commercial / emergency supply lots not enrolled in stability program.
b. ALC-0315 lipid supplier and lot provided here for drug product lots on stability and/or for drug product lots included in the integrity assay acceptance criterion reassessment. All shaded cells in this column used Croda with lot numbers [redacted]
c. Lots in bold text correspond to those manufactured using post-nucleotide triphosphate adjustment drug substance batches and ALC-0315 lipid from Avanti, Groton, or post updated purification process at Croda.
NA = Not Applicable.

Statistical analysis of clinical and Commercial / Emergency use lots

Statistical analysis was applied to the release data for the eighty-three commercial scale drug product lots manufactured, and the mean, standard deviation and minus [redacted] from the mean for the integrity release data set was calculated. In addition to assessment of the full commercial / emergency supply drug product data set (n=83), a subset of these lots (n=28) was also assessed. Subset lot inclusion was based on several factors:

-Drug product lots were manufactured from post-nucleotide triphosphate concentration adjusted process 2 drug substance (20Y513C501 and later manufacture). The nucleotide triphosphate concentration adjustments were correlated with higher observed drug substance integrity. (Lots EE8492 and EE8493 were excluded from this subset).

-Only drug product lots manufactured from Croda ALC-0315 that used a revised purification process with a double chromatography operation, (lot numbers ≥ 1796283, see Table 3.2.P.5.6-27), or lots manufactured from Avanti or Groton ALC-0315, were included. Drug product lots manufactured from the earlier Croda ALC-0315 lots using the purification process with a single chromatography operation were excluded.

Both inclusion criteria, drug product manufactured only from post-nucleotide triphosphate concentration adjusted process 2 drug substance and from ALC-0315 manufactured by post-updated purification process by Croda, or by Avanti or Groton, enable drug product material RNA integrity levels at or above [redacted]. The statistical assessment occurred at a "point in time" with the available drug product lots and since then multiple additional lots have been manufactured, including those manufactured with lipid from additional suppliers. The lots included in the statistical assessment, however, are fully representative of the process as performed in the global environment.

The results of the statistical assessment of the data to determine a one-sided limit ([redacted] for the commercial / emergency supply lots (n=83), for the selected subset of these lots (n=28) and (for comparison) for the Clinical lots (BCV40420-A through EE3813 (BCV40820-P), n=10) are summarized in Table 3.2.P.5.6-28

Table 3.2.P.5.6-28. BNT162b2 Drug Product Release Data for Intact RNA: Statistical Analysis

Description	Commercial / Emergency Supply	Commercial / Emergency Supply Subset[a]	Clinical
N(# of lots):		[redacted]	
Mean:			
SD:			
Min, Max:			
[redacted]			

a. Excludes DP manufactured from pre-nucleotide optimization process 2 DS batches, and DP manufactured from early lots of Croda ALC-0315 lipid (correlated with LMS formation).

When the drug product data set is limited to the subset of lots indicated, the calculated one-sided lower limit from the [redacted] calculation is raised from [redacted] intact RNA to [redacted]

The mean values for the full commercial / emergency supply data set and the associated subset are very similar, and the mean for the clinical /clinical supply lots is slightly higher.

The proposed acceptance criterion for RNA integrity in drug product is shown in Table 3.2.P.5.6-29. The criterion of [redacted] intact RNA at release, and [redacted] on stability and at point of use assure vaccine quality as well as the ability of the vaccine to be distributed and used for longer periods at 2-8°C.

Table 3.2.P.5.6-29. Commercial Release and Stability Acceptance Criterion for RNA Integrity

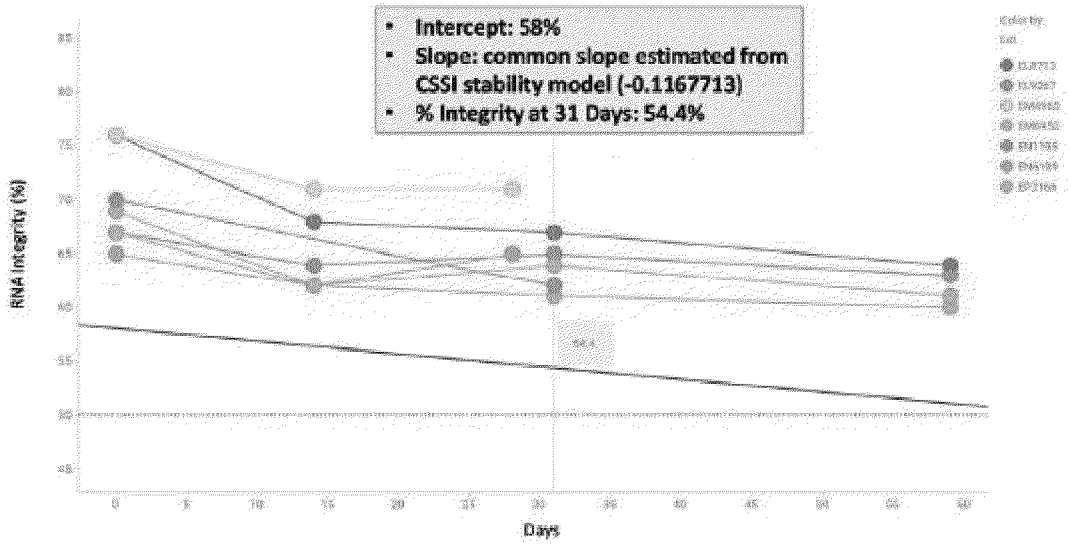
Quality Attribute	Acceptance Criterion
RNA Integrity	[redacted] intact RNA (release) [redacted] intact RNA (stability)

Evaluation of RNA integrity upon drug product storage at 2 – 8 °C

While drug product stability studies to date demonstrate that there is no significant trend (decrease) observed at recommended long-term storage (Section 3.2.P.8.1 Stability Summary and Conclusion), there is a decrease in intact RNA observed at 2 – 8 °C (Section 3.2.P.8.3 Accelerated). To allow for up to 31 days storage at 2 – 8 °C at the point of use, the release acceptance criterion has been tightened to [redacted] intact RNA, thereby enabling the improved storage for pandemic dosing. This acceptance criterion reflects consideration of the [redacted] statistically determined lower limit of [redacted], as well as the evidence of real world efficacy (presented below) for drug product lots at or above [redacted] integrity, and the rate of integrity loss in drug product when stored at 2 – 8 °C for up to 31 days. The acceptance criterion for RNA integrity in drug substance has been set to [redacted] in part to ensure that following drug product manufacture, the level of intact RNA is at or above [redacted]

The rate of decrease in intact RNA for drug product stored at 2 – 8 °C has been determined for the lots representing those in the limited lot subset (N=28) for which RNA integrity was assessed. (Stability lots (N=7) are identified with bolded X's in Table 3.2.P.5.6-27, within the "stability" column). The decrease in RNA integrity across 31 days storage at 2 – 8 °C was determined by calculating a common slope for the fully representative lots of drug product stored for up to 2 months at 2 – 8 °C. Figure 3.2.P.5.6-4 shows the stability data plotted for the seven representative lots along with the common slope determined from the combined data. The black line represents the rate of decrease calculated across 2 months, starting at [redacted] RNA integrity (release limit) and decreasing to [redacted] RNA integrity at 31 days, reflecting the calculated slope of -0.1167713 %RNA integrity/day. Based on the current data set, for drug product lots with minimally [redacted] RNA integrity there is 95% confidence that following 31 days storage at 2 – 8 °C the RNA integrity will be at or above 50%.

Figure 3.2.P.5.6-4. 2 – 8 °C Drug Product Stability Trend (Linear Fit) for RNA Integrity



Clinical justification for the end of shelf life RNA integrity acceptance criterion including recently published real world evidence

The range of intact RNA values determined for the commercial / emergency use supply (both full and limited sets) drug product is predominantly overlapping with that for the clinical data set, and the specification of [redacted] is tighter than [redacted] of the clinical lots. In addition to the clinical data set, support for the proposed RNA integrity specification is provided by a recently published real world study as well as an evaluation of the comparability of capped-intact RNA (the product of the percent 5'- cap and the percent RNA integrity) for clinical and commercial / emergency use supply.

Dajan et al. (N Engl J Med)[1] published an evaluation of vaccine efficacy in a real world setting in Israel which demonstrated high effectiveness of the vaccine, similar to the clinical trial results. A review of lots distributed to Israel in the vaccine time window for this study provides additional clinical support for RNA integrity at [redacted], since the RNA integrity of the lots range from [redacted] (lot [redacted]) to 73% (Table 3.2.P.5.6-2). As previously described, a specification on the intact RNA provides control over both the fragment and late migrating species, since all peaks migrating before and after the intact RNA are integrated, and the reported peak area % of intact RNA is lowered by both the fragment and late migrating species peaks. The late migrating species found in the Israel lots ranges from less than the LOQ to 16% (Table 3.2.P.5.6-2).

In addition to the real-world evidence published by Dajan, et al., an evaluation of the capped-intact levels of the RNA provide additional support of the suitability of the proposed RNA integrity commercial specification. Vaccine efficacy relies on translation of the protein antigen in vivo, and this is assured by full length and capped RNA, known as the capped-intact level. The commercial / emergency use process results in a consistently higher % 5'- cap compared to clinical lots and the proposed 5'- cap commercial specification has been further tightened to [redacted] at the drug substance (Section 3.2.S.4.1 Specification), where it is controlled. The clinical drug substance 5'-cap ranges between [redacted] (see Table 3.2.P.5.6-30). The tightened 5'-cap limit of [redacted] and the RNA integrity proposed limits assure a capped-intact percentage of [redacted] and [redacted], at release and stability/point of use respectively. This capped-intact percentage is within the clinical range which is [redacted] (Table 3.2.P.5.6-30).

Table 3.2.P.5.6-30. Capped-Intact Values for Clinical Lots

DP Batch	BCV40420-A	BCV40620-A	BCV40620-D	BCV40720-A	BCV40720-P / ED3938	BCV40820-P / EE3813
DS Batch	*R427-P020.2-DS*	*R438-P020.2-DS*	*R438-P020.2-DS*	*R443-P020.2-DS*	*R443-P020.2-DS*	*R445-P020.2-DS*
DS Integrity release (%)		[redacted]				
DS 5'-Cap (%)						
DS Capped-Intact (%)						
DP Integrity release (%)						
DP 5'-Cap (%)						
DP Capped-Intact (%)						
DP* Capped-Intact (%)						

*using DS capped value

Overall, the proposed RNA integrity specification ensures vaccine efficacy and manufacturability, and the separate release and point of use limit ensure vaccine access and distribution including more attainable refrigerated storage.

Assessor´s comments

Information provided in sections 3.2.P.5.1 and 3.2.P.5.6 addresses SO2a, SO2d and SO2e regarding the DP. SO2f is addressed in variation EMEA/H/C/005735/II/0054/G.

3.2.P.5.1 Specifications

An updated specifications document has been provided including revised specifications for the drug product at release and throughout shelf-life. Tightening of the acceptance criteria is proposed for osmolality, LNP size, RNA encapsulation, RNA content and lipids content (ALC-0315, ALC-0159, DSPC, cholesterol). All the other parameters remain unchanged from the present and approved drug product specifications document including the acceptance criteria for potency/in-vitro expression, RNA integrity and LNP polydispersity. Separate acceptance criteria for release and on stability are proposed for the LNP size and the RNA integrity.

3.2.P.5.6 Justification of specifications

An updated document has been provided including the justifications for the revised and the unchanged acceptance criteria in the drug product specifications document. A summary of release data for 83 commercial scale DP batches has been provided.

The revised acceptance criteria proposed by the applicant for osmolality, LNP size, RNA encapsulation, RNA content and lipids content (ALC-0315, ALC-0159, DSPC, cholesterol) are all found sufficiently justified and acceptable. The justification is at large based upon development and manufacturing experience (83 commercial DP-batches), ongoing stability studies as well as clinical experience.

The revised acceptance criteria are the following:

- Osmolality: [redacted] mOsmol/kg (from present: [redacted] mOsm/kg)

- LNP size: [redacted] nm (release); [redacted] nm (stability) (from present: [redacted] nm for both release and stability)
- RNA encapsulation: [redacted] (from present: [redacted])
- RNA content: [redacted] mg/mL (from present: [redacted] mg/mL)
- ALC-0315 content: [redacted] mg/mL (from present: [redacted] mg/mL)
- ALC-0159 content: [redacted] mg/mL (from present: [redacted] mg/mL)
- DSPC content: [redacted] mg/mL (from present: [redacted] mg/mL)
- Cholesterol content: [redacted] mg/mL (from present: [redacted] mg/mL)

The justification provided for no change of the DP specifications for all the other quality attributes RNA integrity ([redacted] intact RNA at release; [redacted] intact RNA at stability) and LNP polydispersity ([redacted]) are found acceptable. Additionally, it should be noted that, since the conditional approval of the MAA, the acceptance criteria for the RNA integrity has recently been tightened for both DS and DP at release and shelf-life in the approved variation EMEA/H/C/005735/IB/0031/G.

SO2e and REC10 - Potency/in-vitro expression (IVE) assay:

Additionally, assay variability was further investigated by trading data from DP control samples and a short summary of the related results are provided, which, although seem to support the suitability of the method as a potency indicator, are yet considered too limited to allow for a proper evaluation. Additional details on the performed study and more comprehensive data sets should be provided.

The justification provided for not changing the acceptance criterion in the for potency/in-vitro expression ([redacted] cells positive) in DP the specification is currently based on data obtained from 83 DP batches, manufactured between August 5th 2020 and January 28th 2021, i.e. before the implementation of the changes expected to improve assay robustness and performance. Furthermore, from the data presented in 3.2.P.5.6, only 4 out of 83 batches manufactured early in the development process show potency values below [redacted], which is the lower range for the small-scale clinical batches used in comparability studies provided in the original dossier, and below the lower range ([redacted] for the batches used post-approval in clinical studies, provided in the present variation. Therefore, the specification for the in vitro expression is not yet considered sufficiently justified and should be revised including/based on batches tested after implementation of method improvements.

- A detailed description of the study performed to investigate potency assay variability should be provided, including more detailed summaries of the validation results obtained. Similar information should be provided for the studies performed to investigate assay variability of different replication strategies. The updated study should be included in Module 3.2.P.5.3 of the dossier, for example as an addendum to the current validation report.
- The current specification for the in vitro expression ([redacted] cells positive) is not yet considered sufficiently justified and should be revised including/based on batches tested after implementation of method improvements. If early batches showing low values for potency still will be included in the justification of the acceptance criteria the representativity of these batches should be further addressed.

SO2a, SO2e and REC10 partly remains.

SO2f – Evaluation of lipid-related impurities: The response to SO2f has been submitted in variation EMEA/H/C/005735/II/0054/G (a grouped Type II variation to fulfil SO4, SO5 and SO2f).

6.2.2. Stability summary and conclusion (3.2.P.8.1)

The initial commercial shelf life of the BNT162b2 drug product is 6 months when stored at the intended storage condition of -90 to -60 °C. The initial shelf life is based on the currently available data from stability studies utilizing material from process performance qualification lots, emergency supply lots, clinical lots and one non-clinical lot of drug product. The stability data generated to date on the emergency supply and process performance qualification lots also support an additional storage condition at -20 ± 5°C for up to 2 weeks, as well as short term storage at 5 ± 3°C for up to one month (within the 6 month shelf life).

Additionally, supportive stability studies are also being presented for two clinical BNT162b1 lots. At this time, process performance lots have been enrolled in formal stability programs and available data is provided.

Drug product stability lots have been enrolled in stability programs and are being monitored in accordance with the approved protocols. All testing to date has been performed using analytical methodology and phase appropriate specifications in place at time of testing. The analytical procedures used in the stability programs were developed to monitor the composition, strength, purity, safety and general quality attributes of the drug product.

Drug Product Shelf Life at Recommended Storage Temperature

The initial shelf life of the BNT162b2 is 6 months when stored at the long term storage condition of -90 to -60 °C. The shelf life claim is based on up to 9 months of available stability data for clinical and non-clinical drug product lots, up to 6 months of available stability data for the emergency supply and/or process performance qualification lots and up to 9 months of available supportive BNT162b1 clinical stability lot data, along with scientific rationale and the understanding of the mRNA platform. All drug product lots enrolled in the stability studies are considered to be predictive of the stability of the commercial materials based on comprehensive comparability assessments performed during development.

Additionally, the stability data generated to date on the emergency supply and process performance qualification lots also support an additional storage condition at -20 ± 5 °C for up to 2 weeks, as well as short term storage at 5 ± 3°C for up to one month (within the 6 month shelf life). This is based on current available stability data for the accelerated conditions of -20 ± 5 °C and 5 ± 3°C on emergency supply and process performance qualification lots.

In-use Period of Drug Product

The initial in-use period for the thawed, undiluted vial is room temperature for not more than 2 hours (Section 3.2.P.2.6 Compatibility). Formal thermal cycling stability studies have been initiated on both emergency use lots and PPQ lots in order to further support the in-use period. Available data from these studies is provided in Section 3.2.P.8.3 Thermal Stress and Cycling. The in-use shelf life of undiluted and diluted vials is defined in Section 3.2.P.2.6 Compatibility.

Stability Batches and Studies

The stability program is designed to follow ICH guidelines for stability of drug product (ICH Guideline Q1A: Stability Testing of New Drug Substances and Products; ICH Guideline Q5C: Quality of Biotechnological Products, Stability Testing of Biotechnological/Biological Products). To date, 8 emergency supply lots, 30 process performance qualification/emergency use lots, 7 clinical lots, 1 non-clinical lot and 2 supportive BNT162b1 clinical lots have been placed on stability and stored under long term, accelerated, and thermal stress conditions. The drug product lots placed on stability, to date, were

packaged in glass vials, which are the comparable to the commercial packaging or representative of commercial packaging (for early non-clinical and clinical drug product studies).

Both the clinical and non-clinical drug product lots manufactured by Polymun Scientific using the BNT162b2 construct are considered to be predictive of the stability of the commercial materials based on comprehensive comparability assessments performed during development. The emergency supply lots were manufactured using the commercial process.

As both BNT162b2 and BNT162b1 materials were under clinical development at the same time, it was considered whether available stability data for BNT162b1 could provide additional support for the establishment of shelf life for BNT162b2 drug product. Since the difference between the two constructs is the length of the mRNA (BNT162b2 RNA: 4283 nucleotides; BNT162b1 RNA: 1262 nucleotides) it is reasonable that once encapsulated in the LNPs and processed to DP, quality attributes may be impacted in a similar fashion for both constructs during storage. Therefore, drug product lots manufactured by Polymun Scientific using the BNT162b1 construct are considered predictive of the stability of the commercial materials.

A summary of all drug product lots on stability studies and current available stability data are shown in Table 3.2.P.8.1-1. At this time, stability studies are on-going. Data from these studies will be used to confirm the initial shelf life of the drug product. Further information on confirmation and extension of the drug product shelf life is discussed in Section 3.2.P.8.1.7.

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
FC9873 (Allergopharma/Pfizer Puurs)	May 2021	Stability, Commercial Supply, Process performance qualification	Long Term	-90 to -60 °C	Release	To be started
			Accelerated	-20 ± 5 °C	Release	To be started
			Accelerated	5 ± 3 °C	Release	To be started
FC9877 (Allergopharma/Pfizer Puurs)	May 2021	Stability, Commercial Supply, Process performance qualification	Long Term	-90 to -60 °C	Release	To be started
			Accelerated	-20 ± 5 °C	Release	To be started
			Accelerated	5 ± 3 °C	Release	To be started
FC9880 (Allergopharma/Pfizer Puurs)	May 2021	Stability, Commercial Supply, Process performance qualification	Long Term	-90 to -60 °C	Release	To be started
			Accelerated	-20 ± 5 °C	Release	To be started
			Accelerated	5 ± 3 °C	Release	To be started
FC9909 (Allergopharma/Pfizer Puurs)	May 2021	Stability, Commercial Supply, Process performance qualification	Long Term	-90 to -60 °C	Release	To be started
			Accelerated	-20 ± 5 °C	Release	To be started
			Accelerated	5 ± 3 °C	Release	To be started
SCDD2 (Polymun/Novartis)	May 2021	Stability, Commercial Supply, Process performance qualification	Long Term	-90 to -60 °C	Release	Ongoing
			Accelerated	-20 ± 5 °C	Release	Ongoing
			Accelerated	5 ± 3 °C	Release	Ongoing
SCDN1 (BioNTech Marburg/Novartis)	May 2021	Stability, Commercial Supply, Process performance qualification	Long Term	-90 to -60 °C	Release	To be started
			Accelerated	-20 ± 5 °C	Release	To be started
			Accelerated	5 ± 3 °C	Release	To be started
SCEL5 (mibe/Novartis)	May 2021	Stability, Commercial Supply, Process performance qualification	Long Term	-90 to -60 °C	Release	To be started
			Accelerated	-20 ± 5 °C	Release	To be started
			Accelerated	5 ± 3 °C	Release	To be started
EW6126 (Pfizer Puurs)	May 2021	Stability, Commercial Supply, Process performance qualification	Long term	-90 to -60 °C	Release/T=0	On-going
EY3860 (Pfizer Puurs)	April 2021	Stability, Commercial Supply, Process performance qualification	Long term	-90 to -60 °C	Release	On-going

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EY5422 (Pfizer Puurs)	April 2021	Stability, Commercial Supply, Process performance qualification	Long term	-90 to -60 °C	Release	On-going
EW5279 (BioNTech Marburg/Pfizer, Puurs	March 2021	Stability, Commercial supply, Process performance qualification	Long Term	-90 to -60 °C	Release	On-going
			Accelerated	-20 ± 5 °C	1 month	Complete
EW6327 (BioNTech Marburg/Pfizer, Puurs	March 2021	Stability, Commercial supply, Process performance qualification	Long Term	-90 to -60 °C	Release	On-going
			Accelerated	-20 ± 5 °C	1 month	Complete
EW6326 (BioNTech Marburg/Pfizer, Puurs	April 2021	Stability, Commercial supply, Process performance qualification	Long Term	-90 to -60 °C	Release	On-going
			Accelerated	-20 ± 5 °C	1 month	Complete
1B002A (mibe/Baxter)	March 2021	Stability, Commercial supply, Process performance qualification	Long Term	-90 to -60 °C	Release	On-going
			Accelerated	-20 ± 5 °C	Release	On-going
			Accelerated	5 ± 3 °C	Release	On-going
1B003A (mibe/Baxter)	March 2021	Stability, Commercial supply, Process performance qualification	Long Term	-90 to -60 °C	Release	On-going
			Accelerated	-20 ± 5 °C	Release	On-going
			Accelerated	5 ± 3 °C	Release	On-going
1B004A (mibe/Baxter)	March 2021	Stability, Commercial supply, Process performance qualification	Long Term	-90 to -60 °C	Release	On-going
			Accelerated	-20 ± 5 °C	Release	On-going
			Accelerated	5 ± 3 °C	Release	On-going
1D020A (Marburg/Baxter)	May 2021	Stability, Commercial supply, Process performance qualification	Long Term	-90 to -60 °C	Release	On-going
			Accelerated	-20 ± 5 °C	Release	On-going
			Accelerated	5 ± 3 °C	Release	On-going
ER1741 (Re-filtration lot Pfizer, Puurs)	April 2021	Emergency Supply, Stability	Long Term	-90 to -60 °C	Release/T=0	On-going

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EN6199 (Pfizer, Kalamazoo Line 18)	February 2021	Stability, Emergency Supply[a], Process performance qualification	Long Term	-90 to -60 °C	3 months	On-going
			Accelerated	-60 to -30 °C	Release	On-going
			Accelerated	-20 ± 5 °C	3 months	On-going
			Accelerated	5 ± 3 °C	3 months	On-going
EL3249 (Pfizer, Kalamazoo Line 18)	December 2020 (January 2021 for Thermal Cycling Study)	Stability, Clinical, Emergency Supply[a], Process performance qualification	Long Term	-90 to -60 °C Upright Vials	3 months	On-going
				-90 to -60 °C Inverted Vials	Release	On-going
			Accelerated	-20 ± 5 °C Upright Vials	3 months	On-going
				-20 ± 5 °C Inverted Vials	1 month	On-going
			Accelerated	5 ± 3 °C Upright Vials	3 months	On-going
				5 ± 3 °C Inverted Vials	1 month	On-going
			Thermal Cycling	Thermal Cycling: 1 week at -90 to -60°C , followed by 2 weeks at -20 ± 5 °C, 4 weeks at 2 to 8°C and 1 week at 25 ± 2 °C/60 ± 5% RH.	8 weeks	Complete
EL9267 (Pfizer, Kalamazoo, Line 8)	January 2021	Stability, Emergency Supply[a], Process performance qualification	Long Term	-90 to -60 °C	3 months	On-going
			Accelerated	-60 to -30 °C	Release	On-going
			Accelerated	-20 ± 5 °C	3 months	On-going
			Accelerated	5 ± 3 °C	3 months	On-going
EL3248 (Pfizer, Kalamazoo, Line 8)	December 2020	Stability, Clinical, Emergency Supply[a], Process performance qualification	Long Term	-90 to -60 °C Upright Vials	3 months	On-going
				-90 to -60 °C Inverted Vials	Release	On-going
			Accelerated	-20 ± 5 °C Upright Vials	3 months	On-going
				-20 ± 5 °C Inverted Vials	1 month	On-going
			Accelerated	5 ± 3 °C Upright Vials	3 months	On-going
				5 ± 3 °C Inverted Vials	1 month	On-going
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month (complete)	On-going
			Thermal Stress	30 ± 2 °C/ 65 ± 5 % RH	1 month (complete)	On-going
EN6198 (Pfizer, Kalamazoo Line 18)	March 2021	Stability, Emergency Supply[a], Process performance qualification	Photostability	Dark Control and Light Exposed		Complete
EL9266 (Pfizer, Kalamazoo)	February 2021	Stability, Emergency Supply[a], Process performance qualification	Thermal Cycling	Thermal Cycling: Ultra frozen vials are placed at -20 ± 5 °C for 4 weeks and then moved to 2 to 8°C for 12 weeks. Samples will be pulled for testing every 2 weeks throughout protocol.	16 weeks	On-going

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EL8723 (Pfizer, Puurs)	January 2021 (February 2021 for Thermal Cycling Study 1 and April 2021 for Thermal Cycling Studies 2 & 3)	Stability, Clinical, Emergency Supply[a], Process performance qualification	Long Term	-90 to -60 °C	3 months	On-going
			Accelerated	-60 to -30 °C	Release	On-going
			Accelerated	-20 ± 5 °C	3 months	On-going
			Accelerated	5 ± 3 °C	3 months	On-going
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month (complete)	On-going
			Thermal Stress	30 ± 2 °C/ 65 ± 5 % RH	1 month (complete)	On-going
			Thermal Cycling	Thermal Cycling 1: Ultra frozen vials are placed at -20 ± 5 °C for 4 weeks and then moved to 2 to 8°C for 12 weeks. Samples will be pulled for testing every 2 weeks throughout protocol.	16 weeks	On-going
			Thermal Cycling	Thermal Cycling 2: Ultra frozen vials are placed at -20 ± 5 °C for 4 weeks (1 month) and then moved to -90 to -60 °C for the shelf life of the drug product.	1 month	On-going
			Thermal Cycling	Thermal Cycling 3: Ultra frozen vials are placed at 5 ± 3 °C for 4 weeks (1 month) and then moved to -90 to -60 °C for the shelf life of the drug product.	1 month	On-going
			Thermal Cycling	Ultrafrozen vials were cycled between 25 ± 2 °C/60 ± 5%RH (for 2 to 7 hours) and -90 to -60 °C (for 24 ± 2 hours) for a total of 10 cycles	10 freeze thaw cycles	Complete
EL1491 (Pfizer, Puurs)	December 2020	Stability, Emergency Supply[a], Clinical, Process performance qualification	Long Term	-90 to -60 °C Upright Vials	6 months	On-going
				-90 to -60 °C Inverted Vials	6 months	On-going
			Accelerated	-20 ± 5 °C Upright Vials	6 months	On-going
				-20 ± 5 °C Inverted Vials	6 months	On-going
			Accelerated	5 ± 3 °C Upright Vials	6 months	On-going
				5 ± 3 °C Inverted Vials	6 months	On-going

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EH9899 (Pfizer, Kalamazoo)	November 2020	Stability, Emergency Supply[a]	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-20 ± 5 °C	6 months	On-going
			Accelerated	5 ± 3 °C	3 months	Complete
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month	Complete
EJ1688 (mibe/Pfizer, Puurs)	November 2020	Stability, Emergency Supply[a]	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-20 ± 5 °C	6 months	On-going
			Accelerated	5 ± 3 °C	3 months	Complete
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month	Complete
EK1768 (Polymun Scientific/Pfizer, Puurs	November 2020 (December 2020 for Thermal Cycling Study)	Stability, Emergency Supply[a], Clinical inventory	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-20 ± 5 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month	Complete
			Thermal Cycling	Thermal Cycling: 2 weeks at -90 to -60°C followed by 4 weeks at -20 ± 5 °C and then 8 weeks at 2 to 8°C. Samples will be pulled for testing every 2 weeks throughout protocol.	14 weeks	Complete
EJ1686 (Polymun Scientific/Pfizer, Puurs)	November2020 (December 2020 for Thermal Cycling Study)	Stability, Emergency Supply[a], Clinical inventory	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-20 ± 5 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month	Complete
			Thermal Cycling	Thermal Cycling: 2 weeks at -90 to -60°C followed by 4 weeks at -20 ± 5 °C and then 8 weeks at 2 to 8°C. Samples will be pulled for testing every 2 weeks throughout protocol.	14 weeks	Complete
EJ1685 (Polymun Scientific/Pfizer, Puurs)	November 2020	Stability, Emergency Supply[a], Clinical inventory	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-60 to -30 °C	6 months	On-going
			Accelerated	-20 ± 5 °C	6 months	On-going
			Accelerated	5 ± 3 °C	3 months	Complete
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
EJ0553 (Polymun Scientific/Pfizer, Puurs)	November 2020	Stability, Emergency Supply[a], Clinical inventory	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-60 to -30 °C	6 months	Complete
			Accelerated	-20 ± 5 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month	Complete
EE8493 (Polymun Scientific/Pfizer, Puurs)	September 2020	Stability, Emergency Supply[a], Clinical inventory	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-60 to -30 °C	6 months	Complete
			Accelerated	-20 ± 5 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
			Thermal Stress	25 ± 2 °C/ 60 ± 5 % RH	1 month	Complete
			Thermal Stress	30 ± 2 °C/ 65 ± 5 % RH	1 month	Complete
EE8492 (Polymun Scientific/Pfizer, Puurs)	September 2020	Stability, Emergency Supply[a]	Long Term	-90 to -60 °C	6 months	On-going
			Accelerated	-20 ± 5 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
EE3813[c] (Polymun Scientific/Pfizer, Puurs)	August 2020	Stability, Clinical	Long Term	-70 ± 10 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
ED3938[d] (Polymun Scientific/Pfizer, Puurs)	August 2020	Stability, Clinical inventory	Long Term	-70 ± 10 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
BCV40720-C (Polymun Scientific)	August 2020	Stability, Clinical	Long Term	-70 ± 10 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
BCV40720-A (Polymun Scientific)	August 2020	Stability, Clinical	Long Term	-70 ± 10 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
BCV40620-E (Polymun Scientific)	July 2020	Stability, Nonclinical	Long Term	-70 ± 10 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete
BCV40620-A (Polymun Scientific)	July 2020	Stability, Clinical	Long Term	-70 ± 10 °C	6 months	Complete
			Accelerated	5 ± 3 °C	3 months	Complete

Table 3.2.P.8.1-1. Summary of On-going Stability Studies

Lot Number	Stability Study Start	Drug Product Batch Use	Study Type	Storage Condition	Data Available	Study Status
BCV40420-A (Polymun Scientific)	May 2020	Stability, Clinical	Long Term	-70 ± 10 °C	12 months	On-going
			Accelerated	-40 ± 5 °C	12 months	On-going
			Accelerated	5 ± 3 °C	6 months	Complete
			Thermal Stress	25 ± 2 °C	4 months	Complete
CoVVAC/270320 (Polymun Scientific)	March 2020	Stability, non-clinical toxicology[b]	Long Term	-70 ± 10 °C	6 months	Complete
			Accelerated	-40 ± 5 °C	3 months	On-going
			Accelerated	5 ± 3 °C	6 months	Complete
Supportive Stability (BNT162b1)						
BCV10420-A (Polymun Scientific)	April 2020	Supportive Stability, Clinical	Long Term	-70 ± 10 °C	12 months	Complete
BCV10320-A (Polymun Scientific)	April 2020	Supportive Stability, Clinical	Long Term	-70 ± 10 °C	12 months	On-going
			Accelerated	-40 ± 5 °C	9 months	On-going
			Accelerated	5 ± 3 °C	6 months	Complete
			Thermal Stress	25 ± 2 °C	3 months	Complete

a. Emergency supply designation applies to US market.
b. -40 °C study started in April 2020.
c. This lot number is equivalent to BCV40820-P
d. This lot number is equivalent to BCV40720-P.
TBD = To be Determined, RH = Relative Humidity

Assessor´s comments

An updated document has been provided including stability information for additional lots at long-term, accelerated and stressed storage conditions as well as thermal cycling and photostability studies.

It should be noted that the DP shelf life was recently extended from 6 months to 9 months at -90 °C to -60 °C in variation EMEA/H/C/005735/IB/0061.

REC20: 6 month stability data at long-term storage conditions (-90 to -60 °C) have been provided for DP PPQ batches manufactured at mibe (EK4242) and Pfizer, Puurs (EL1491). However, since only 3 months stability data are provided at long-term conditions for DP batches manufactured at Polymun (EM4965 and EL7834), the 6 months data are expected to be submitted in a future variation. REC20 is consequently found partially fulfilled.

The provided information in sections 3.2.P.8.1 and 3.2.P.8.3 addresses SO2a and SO2d regarding the DP. REC20 is found partially fulfilled.

6.3. Concluding comments for both DS and DP

Assessor's concluding comments:

The primary purpose of this submission is to fulfil Specific Obligations 1 and 2 (SO1 and SO2). To fulfil SO1, additional characterisation information for the active substance and finished product is provided. To fulfil SO2, the drug substance and drug product release and stability specification acceptance criteria have been reassessed and revised.

Specific Obligation 1: Characterisation of the active substance and finished product

SO1a: Truncated and modified mRNA species

The Applicant has provided additional characterisation data. The potential for truncated transcripts to produce proteins/peptides was further investigated using a cell-free in vitro expression system. However, the method used needs to be appropriately described, including conditions for in vitro reaction, antibodies used and choice of investigated material. Also, additional BNT162b2 batches with different levels of RNA integrity at release are expected to be included in the characterization exercise.

SO1a partly remains.

SO1b: Analysis of the main peak of the RNA integrity test

It is sufficiently demonstrated that the major proportion of fragmented species from one representative Process 1 and one representative Process 2 DS batch contains the 5'-cap but lacks the poly(A) tail.

SO1b is considered solved.

SO1c: Identities of the observed Western Blot (WB) bands obtained by the in vitro expression assay

WB results obtained by three different antibodies, specific for the S1 domain, the receptor binding domain and the S2 domain, respectively, are presented and compared to theoretical masses of the S-protein and the subdomains in glycosylated and non-glycosylated forms. Even though the quality of the anti-S2-antibody WB is considered poor, it is sufficiently justified that the major band monitored by all three WB-antibodies corresponds to the heavily glycosylated S-protein. A protein of size 140 kDa, corresponding to the deglycosylated form of the S-protein, is obtained upon PNGase digestion.

SO1c is considered solved.

Specific Obligation 2: Control strategy

SO2a: The active substance and finished product specifications acceptance limits

For Poly (A) tail length a new method with a qualitative acceptance criterion has been added and for 5'-Cap the criterion has been tightened from [redacted], these are the only changes proposed to the DS specification. The acceptance criterion for RNA integrity was tightened from [redacted] and approved in connection with EMEA/H/C/005735/IB/0031/G (partial fulfillment of SO2a, SO2d). No further tightening is proposed or considered necessary.

SO2a is considered solved from a DS perspective.

For drug product the tightening of the acceptance criteria proposed for osmolality, LNP size, RNA encapsulation, RNA content and lipids content (ALC-0315, ALC-0159, DSPC, cholesterol) are sufficiently justified and found acceptable.

The specification for the in vitro expression is not yet considered sufficiently justified and should be revised including/based on batches tested after implementation of method improvements.

SO2a partly remains from a DP perspective.

SO2b: Poly(A) tail length

The level of details provided for the validation exercise for the new IP-RP-HPLC is considered too limited to allow for a proper assessment. More detailed method validation summaries including test results for individual samples, calculations, chromatograms etc. should be provided to support equal and adequate performance of the test method at all test sites. Module 3.3.S.4.3 of the dossier should be updated accordingly.

SO2b partly remains.

SO2c: Poly(A) tail percentage

The accuracy of the ddPCR method needs to be further addressed in relation to available data indicating a consistent overestimation of % poly(A) tail content, as 9 out 42 batches provided show results in the range of [redacted].

SO2c partly remains.

SO2d: mRNA integrity and polydispersity of the finished product

The justifications provided for no change of the DP specifications for RNA integrity ([redacted] intact RNA at release; [redacted] intact RNA at stability) and LNP polydispersity ([redacted]) are found acceptable. Additionally, it should be noted that, since the conditional approval of the MAA, the acceptance criteria for the RNA integrity has recently been tightened for both DS and DP at release and shelf-life in the approved variation EMEA/H/C/005735/IB/0031/G.

SO2d is considered solved.

SO2e and REC10: Potency/in-vitro expression (IVE) assay

Additional details are still needed to support the suitability of potency method.

SO2e and REC10 partly remains.

SO2d: Lipid-related impurities

See separate assessment report for EMEA/H/C/005735/II/0054/G.

REC20:

Since only 3 months stability data are provided at long-term conditions for DP batches manufactured at Polymun (EM4965 and EL7834), the 6 months data are expected to be submitted in a future variation.

REC20 not fulfilled.

Conclusion:

The Specific Obligations 1 and 2 are only considered as partly solved and the issues below needs to be responded to:

- Information should be provided regarding the settings of the cell-free in vitro translation study. The method used should be appropriately described, including conditions for in vitro reaction, antibodies used, the nature of the positive and negative controls and choice of investigated material. Release data for the batch used in the study should be provided and additional BNT162b2 batches with different levels of RNA integrity at release, ideally having historically low and high levels of RNA integrity, should be included in the characterization exercise. (From SO1a)
- The updated characterisation data included in the response document should be included in Module 3.2.S.2.6 and/or 3.2.S.3 of the dossier. (From SO1)

- The level of details provided for the validation exercise for the new IP-RP-HPLC is considered too limited to allow for a proper assessment. More detailed method validation summaries including test results for individual samples, calculations, chromatograms etc. should be provided to support equal and adequate performance of the test method at all test sites. Module 3.3.S.4.3 of the dossier should be updated accordingly. (From SO2b)
- The accuracy of the ddPCR method needs to be further addressed in relation to available data indicating a consistent overestimation of % poly(A) tail content, as 9 out 42 batches provided show results in the range of [redacted]. (From SO2c)
- The updated DS specification should be provided as replaced documents and not as a new document. Module 3.2.S.4.1 of the dossier should be updated accordingly. (From SO2)
- A detailed description of the study performed to investigate potency assay variability should be provided, including more detailed summaries of the validation results obtained. Similar information should be provided for the studies performed to investigate assay variability of different replication strategies. The updated study should be included in Module 3.2.P.5.3 of the dossier, for example as an addendum to the current validation report. (From SO2e and REC10)
- The current specification for the in vitro expression ([redacted] cells positive) is not yet considered sufficiently justified and should be revised including/based on batches tested after implementation of method improvements. If early batches showing low values for potency still will be included in the justification of the acceptance criteria the representativity of these batches should be further addressed. (From SO2a, SO2e and REC10)
- Since only 3 months stability data are provided at long-term conditions for DP batches manufactured at Polymun (EM4965 and EL7834), the 6 months data are expected to be submitted in a future variation. (REC20)

7. Changes to the Product Information

This grouped variation does not impact the product information.

8. Request for supplementary information

8.1. Major objections

None.

8.2. Other concerns

Quality aspects

1. Information should be provided regarding the settings of the cell-free in vitro translation study. The method used should be appropriately described, including conditions for in vitro reaction, antibodies used, the nature of the positive and negative controls and choice of investigated material. Release data for the batch used in the study should be provided and additional BNT162b2 batches with

different levels of RNA integrity at release, ideally having historically low and high levels of RNA integrity, should be included in the characterization exercise. (From SO1a)

2. The updated characterisation data included in the response document should be included in Module 3.2.S.2.6 and/or 3.2.S.3 of the dossier. (From SO1)
3. The level of details provided for the validation exercise for the new IP-RP-HPLC is considered too limited to allow for a proper assessment. More detailed method validation summaries including test results for individual samples, calculations, chromatograms etc. should be provided to support equal and adequate performance of the test method at all test sites. Module 3.3.S.4.3 of the dossier should be updated accordingly. (From SO2b)
4. The accuracy of the ddPCR method needs to be further addressed in relation to available data indicating a consistent overestimation of % poly(A) tail content, as 9 out 42 batches provided show results in the range of [redacted] (From SO2c)
5. The updated DS specification should be provided as replaced documents and not as a new document. Module 3.2.S.4.1 of the dossier should be updated accordingly. (From SO2)
6. A detailed description of the study performed to investigate potency assay variability should be provided, including more detailed summaries of the validation results obtained. Similar information should be provided for the studies performed to investigate assay variability of different replication strategies. The updated study should be included in Module 3.2.P.5.3 of the dossier, for example as an addendum to the current validation report. (From SO2e and REC10)
7. The current specification for the in vitro expression ([redacted] cells positive) is not yet considered sufficiently justified and should be revised including/based on batches tested after implementation of method improvements. If early batches showing low values for potency still will be included in the justification of the acceptance criteria the representativity of these batches should be further addressed. (From SO2a, SO2e and REC10)
8. Since only 3 months stability data are provided at long-term conditions for DP batches manufactured at Polymun (EM4965 and EL7834), the 6 months data are expected to be submitted in a future variation. (REC20)

9. Assessment of the responses to the request for supplementary information

9.1. Major objections

Quality aspects

None

9.2. Other concerns

Quality aspects

Question 1

Information should be provided regarding the settings of the cell-free in vitro translation study. The method used should be appropriately described, including conditions for in vitro reaction, antibodies used, the nature of the positive and negative controls and choice of investigated material. Release data for the batch used in the study should be provided and additional BNT162b2 batches with different levels of RNA integrity at release, ideally having historically low and high levels of RNA integrity, should be included in the characterization exercise. (From SO1a).

Summary of the MAH's response

The cell-free in vitro translation is performed using the commercially available Rabbit Reticulocyte Lysate (RRL) system, which is provided by Promega. The key components for the in vitro translation reaction are the RRL, the amino acid mixtures as well as a precharged, biotin-labeled, lysine tRNA. During the translation reaction the labeled lysines are incorporated into the nascent protein chain, which enables the visualization of the resulting protein. For that, after completion of the translation reaction, the samples are subjected to a denaturing gel electrophoresis procedure (SDS-PAGE; Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) followed by the transfer to a nitrocellulose membrane via electroblotting. The visualization of the protein is achieved by binding of the biotin-labeled lysines to Streptavidin-Alkaline Phosphatase (Strep-AP) followed by colorimetric detection.

As positive control a luciferase-encoding RNA is used, which is provided within the translation kit and results in a protein band at a size of approximately 60 kDa. As negative control a sample was generated, which was subjected the complete assay workflow without any RNA in the reaction mixture to determine background signals that are not related to the RNA of the test sample.

The RNA that was used in the in vitro translation study was produced as engineering run in the BioNTech Mainz / Rentschler manufacturing node generating representative material according to the commercial process (batch no: 1071509; 37.6 L IVT reaction; 180 L drug substance; expected protein size: approx. 140 kDa). The batch data are presented in Table 1.

Table 1. Batch data of the engineering run 1071509 (BioNTech Mainz/ Rentschler)

Parameter	Result
RNA integrity	[redacted]
dsRNA	[redacted] pg dsRNA / µg RNA
Residual DNA template	[redacted] ng DNA / mg RNA
Identity of encoded RNA sequence	Identity confirmed
Appearance (Coloration)	Not more intensely colored than level 7 of brown (B) color standard
Appearance (Clarity)	[redacted] NTU
Content (RNA concentration)	[redacted] mg/mL
Bioburden	0 CFU / 10 mL
Endotoxins	< 1.0 EU/mL
pH	[redacted]
5´-Cap	[redacted]
Poly(A) tail	[redacted]

To generate different integrity levels, the RNA sample (batch 1071509) was subjected to a thermal degradation procedure, which was stopped for each level after a certain amount of incubation time. After analysis of the RNA integrity, the sample was subjected to the in vitro translation procedure as described above.

The starting material (batch 1071509) is representative of the DS, and the resulting range of RNA integrity after thermal degradation is wider than the range observed in DS batches. Therefore, the thermally degraded material was used in this characterization study.

Of note, no visual difference in the Western blot pattern can be seen in the intensity of the first two quality stages (0 min degradation [redacted] integrity) and 2 min degradation [redacted] integrity)) indicating that no differences in the pattern using RNA in this RNA integrity range can be expected, which would be the case for any additional historical batch.

Assessment of the MAH's response

Additional information regarding the cell-free in vitro translation study has been provided. The study is performed using the commercially available Rabbit Reticulocyte Lysate (RRL) system which is very briefly described. The positive and negative controls have now been identified as a luciferase-encoding RNA, provided within the translation kit and resulting in a protein band at a size of approximately 60 kDa, and a sample subjected to the complete assay workflow without any RNA in the reaction mixture, respectively. Although the information provided with respect to the settings of this study is limited, it could be considered sufficient to support its relevance for the intended purpose.

Details on the batch used for the cell-free in vitro translation study are now submitted: This is an engineering run batch stated to be representative for the DS, with values of [redacted] for RNA integrity, [redacted] for 5'-Cap and [redacted] for Poly(A) tail. Indeed, thermal degradation studies as presented in the original variation application do not show any visual difference in the western blot pattern. However, these results are not informative of whether batches with different mRNA integrity at release and therefore possible different mRNA variants might have different western blot patterns after in vitro translation in the cell-free system. Therefore, additional data using the cell-free *in vitro* translation system are considered needed to further support product characterization. Although requested, these data have not been provided and are still expected to fully resolve this issue (REC).

Conclusion

Solved with the following recommendation:

The MAH should complement the characterization exercise using the cell-free *in vitro* translation system with additional tozinameran batches (at least three) representing the range of low to high RNA integrity levels at release.

Question 2

The updated characterisation data included in the response document should be included in Module 3.2.S.2.6 and/or 3.2.S.3 of the dossier. (From SO1)

Summary of the MAH's response

The updated characterization data included in the Specific Obligation 1 response document has been included in the revised Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics.

Assessment of the MAH's response

The updated characterization data included in the Specific Obligation 1 response document has been included in the dossier.

Conclusion

The issue is resolved.

Question 3

The level of details provided for the validation exercise for the new IP-RP-HPLC is considered too limited to allow for a proper assessment. More detailed method validation summaries including test results for individual samples, calculations, chromatograms etc. should be provided to support equal and adequate performance of the test method at all test sites. Module 3.3.S.4.3 of the dossier should be updated accordingly. (From SO2b)

Summary of the MAH's response

The reports documenting the validation of the IP-RP-HPLC method at Pfizer Analytical Research and Development, Pfizer Global Supply, Andover, MA, and BioNTech, Marburg are provided in the 3.2.S.4.3 IP-RP-HPLC of the dossier, and the validation summaries originally submitted in SO1/SO2 have been removed.

Assessment of the MAH's response

As requested, new validation reports with additional information have been provided for the new method to evaluate poly(A) tail length. Module 3.3.S.4.3 of the dossier have been updated accordingly.

Conclusion

The issue is resolved.

Question 4

The accuracy of the ddPCR method needs to be further addressed in relation to available data indicating a consistent overestimation of % poly(A) tail content, as 9 out 42 batches provided show results in the range of [redacted]. (From SO2c)

Summary of the MAH's response

The method performance (accuracy and reproducibility) of the ddPCR method was determined during method validation to be [redacted] accuracy and [redacted] RSD, determined at target level ([redacted] measured over 8 instances (VAL100146747 – Report for the validation of test method TM100010379: Quantitation of Poly A Tail in BNT162B2 mRNA PF-07305885 (Drug Substance) using ddPCR technology).

The distribution of poly(A) tail content among the 42 batches described previously, as determined using the ddPCR method, is shown in Figure 1, with bars representing the number of test results within a given range and lines representing a normal distribution of the data. A similar overall distribution was observed in analytical reference material lots 20Y513C201-RM (Figure 2) and 20Y513C701-WRM (Figure 3), which were measured across 102 and 90 instances, respectively. For comparative purposes, the three distributions are overlaid in Figure 4. Consistent with the results from method validation and the

distribution in measured poly(A) tail content across reference materials, the range in drug substance release values is not unexpected. When assuming the true Poly(A) tail result for all DS batches is [redacted] and the assay method precision (assay standard deviation) is [redacted] which is the same as the assay results summarized in Figure 1, then based on the normal distribution statistically it is expected to have [redacted] of the assay data above [redacted] or about 4 out of 42, which is close to the DS batches analysis results that there are 5 batches with results are at or above [redacted] (or in range of [redacted]). Therefore, the accuracy of the ddPCR method is considered to be suitable for drug substance release and stability testing.

Figure 1. Distribution of poly(A) tail content in 42 DS batches

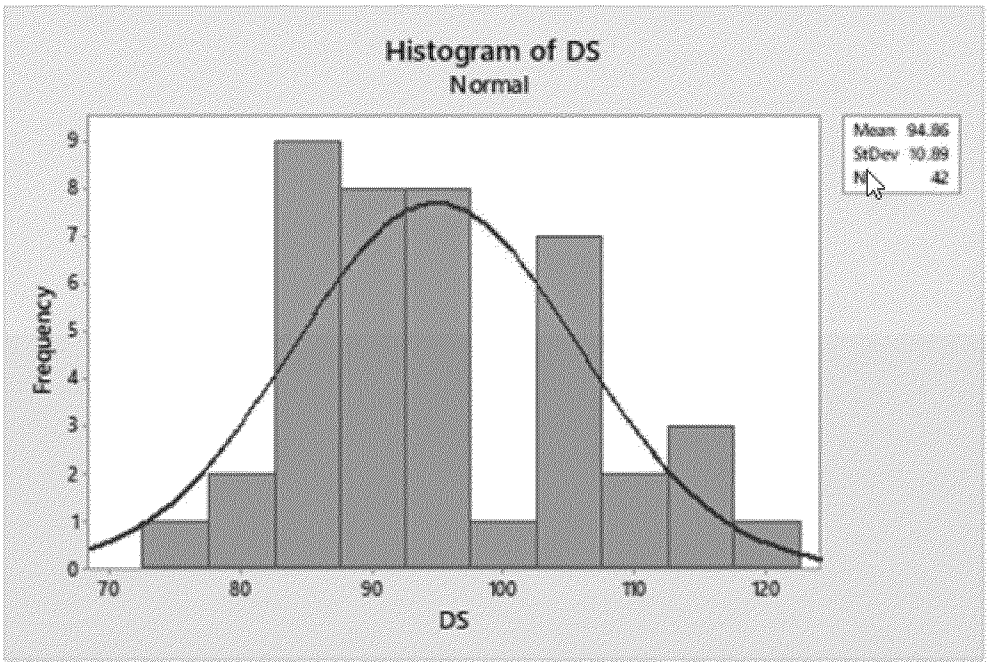


Figure 2. Poly(A) tail content measured in batch 20Y513C201-RM over 102 instances

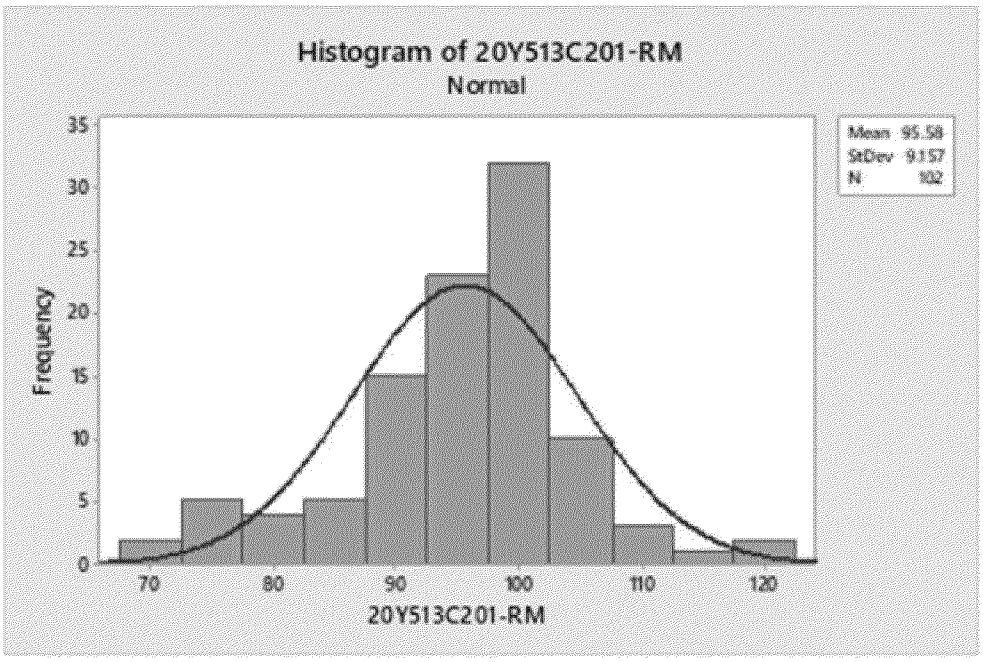


Figure 3. Poly(A) tail content measured in batch 20Y513C701-WRM over 90 instances

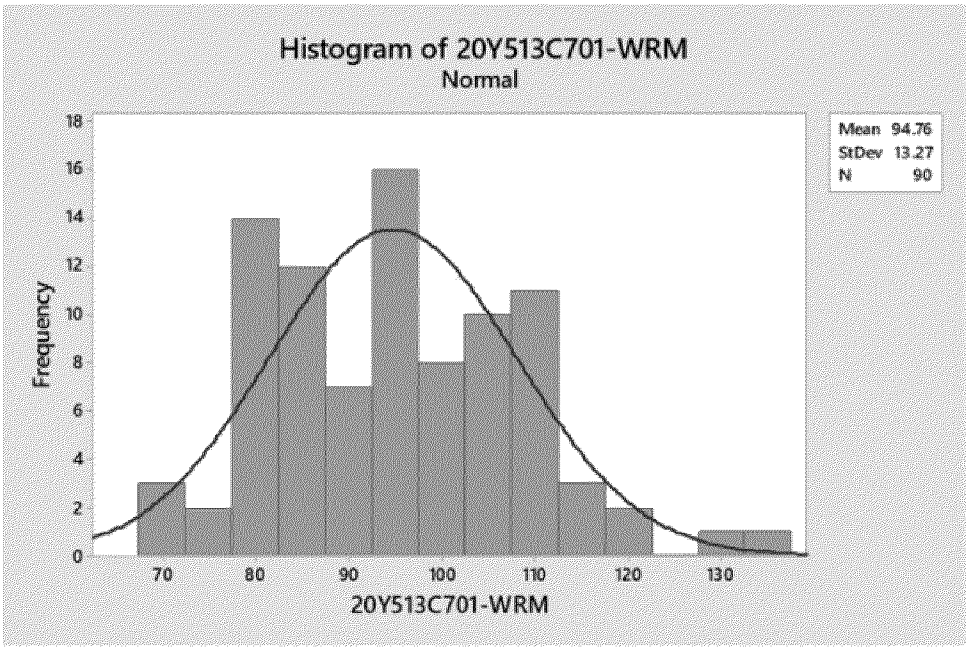
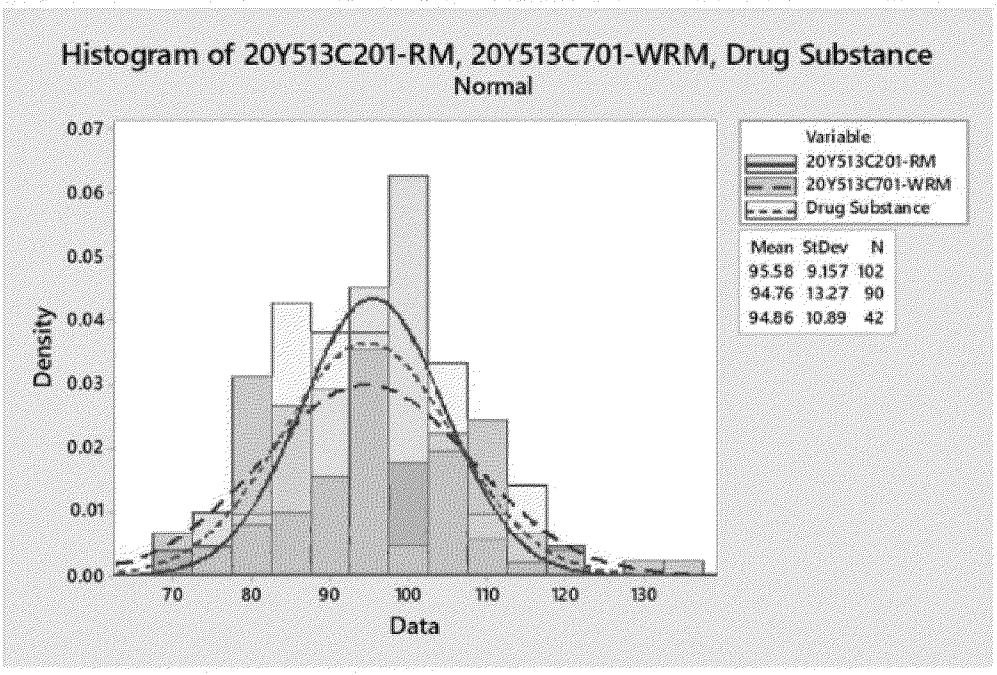


Figure 4. Overlay of Reference Material and Historical DS Batch Data



Assessment of the MAH's response

Additional data and graphs are provided on the distribution of the poly(A) tail content among the 42 batches included in the original variation application as well as for two analytical material reference lots, analyzed across 102 and 90 instances, respectively. Based on these data, the statistical distribution is

expected to have [redacted] of measurements above [redacted] which is the value observed in the analysis of the 42 batches previously described.

Issue solved.

Question 5

The updated DS specification should be provided as replaced documents and not as a new document. Module 3.2.S.4.1 of the dossier should be updated accordingly. (From SO2)

Summary of the MAH's response

Module 3.2.S.4.1 Specifications from EMEA/H/C/005735/II/0056/G (sequence 0144) is confirmed to be the version that will be maintained. All other versions will be replaced with this submission.

Assessment of the MAH's response

The specification provided in sequence 0144 is still provided as a new document and not as a replaced document as expected. However, it is understood that this will be corrected in the closing sequence.

Conclusion

The response is found acceptable and the issue ***will be resolved in the closing sequence.***

Question 6

A detailed description of the study performed to investigate potency assay variability should be provided, including more detailed summaries of the validation results obtained. Similar information should be provided for the studies performed to investigate assay variability of different replication strategies. The updated study should be included in Module 3.2.P.5.3 of the dossier, for example as an addendum to the current validation report. (From SO2e and REC10)

Summary of the MAH's response

The original validations were executed using a [redacted] primary antibody dilution with a replication strategy of [redacted] assay instances run in quadruplicate per instance. Supplemental validations were executed using a [redacted] dilution of primary antibody and a streamlined replication strategy, [redacted] assay instances run in duplicate per instance. The full validation report, including supplemental validation data, was submitted in 3.2.P.5.3 Validation of Analytical Procedure – Cell Based Flow Cytometry on 30 September 2021 as part of EMEA/H/C/005735/II/0075/G (seq 0199). Statistical analysis performed on the original and supplemental validation data demonstrated that the streamlined replication strategy may be implemented with minimal impact on overall assay variability.

Evaluation of potency assay variability is based on data generated in three Pfizer labs, ARD-Andover, ARD-St Louis, and PGS-Grange Castle using the in vitro expression (IVE) method implemented with [redacted] instances per assay and [redacted] wells per instance testing strategy. A total of 235 instances of drug product control (DPC) and 918 instances of test sample (TS) data were analyzed. Data is shown in Table 1 and Table 2. The statistical variance component analysis was used to quantify the variability due to different sources including lab, analyst, experiment, instance, and well for DPC. The results are shown in Table 1. The same statistical analysis was applied to TS data and the variability due to instance and well were quantified respectively, which are shown in Table 2. Since instance-to-instance variability and well-to-well variability from TS is slightly higher than that of DPC, the calculation of total assay variability (Total assay

SD) used the analyst and experiment variability from DPC data and instance and well variability from TS data as worst case scenario, based on the following formula

$$\text{Total assay SD}=\sqrt{var(Analyst)+var(Experiment)+\frac{var(Instance)}{n(Instance)}+\frac{var(Well)}{n(Instance)*n(Well)}}$$

The total IVE assay SD is determined to be 13.53%. The statistical software R 3.6.1 was used for the above statistical analysis.

Table 1. IVE Variance Component Analysis by DPC Results

Source of Variability	Standard Deviation (SD, %)
Lab	[redacted]
Analyst	
Experiment within analyst	
Instance within experiment	
Well within Instance	

Table 2. IVE Variance Component Analysis by TS Results

Source of Variability	Standard Deviation (SD, %)
Instance within experiment	[redacted]
Well within Instance	

Based on the variance component estimates for DPC and TS data above, the total assay variability for two new replication strategies evaluated ([redacted] instances per assay, [redacted] wells per instance, and [redacted] instances per assay, [redacted] per instance) was also calculated using the same formula above. The resulting total assay SD is very close to the current total assay SD, [redacted] (Table 3).

Table 3. Total Assay Variability of Different Replication Strategies

Assay Replication Strategy	Total Assay Standard Deviation (SD, %)
[redacted] instances per assay, [redacted] wells per instance [a]	[redacted]
[redacted] instances per assay, [redacted] wells per instance	
[redacted] instances per assay, [redacted] wells per instance	

a. Current assay replication strategy

The same statistical analysis was performed for the validation results. Same drug product lot was used as DPC (63 instances) and Sample (66 instances at the target concentration) during the validation. The total assay variability for validation results (DPC and Sample at the target concentration) is [redacted] (**Error! Reference source not found.**).

Table 4. Variability Analysis of Validation Results

Source of Variability	Standard Deviation (SD, %)
Total Assay	[redacted]
Instance within experiment	
Well within Instance	

Assessment of the MAH's response

Additional details of the study performed to investigate potency assay variability especially concerning different replication strategies have been provided. Although in the format of very short summaries, the submitted data support a similar assay variability irrespective to the replication strategy, i.e between [redacted] and [redacted] assay SDs for assays replications strategies of [redacted] instances per assay and [redacted] wells per instance; [redacted] instances per assay and [redacted] wells per instance; and [redacted] instances per assay and [redacted] wells per

instance. The approach is considered adequate. It is expected that the dossier will be updated with the provided data in the closing sequence.

Conclusion

The response is found acceptable, and the issue ***will be resolved in the closing sequence.***

Question 7

The current specification for the in vitro expression ([redacted] cells positive) is not yet considered sufficiently justified and should be revised including/based on batches tested after implementation of method improvements. If early batches showing low values for potency still will be included in the justification of the acceptance criteria the representativity of these batches should be further addressed. (From SO2a, SO2e and REC10)

Summary of the MAH's response

The specification for the in vitro expression assay has been revised to [redacted] S1+ cells for release and stability based on 654 released lot data after implementation of method improvement. The method improvement was implemented after February 2021. Early lots prior to the implementation of the method improvement were not included in the re-assessment. Justification of the revision is provided in 3.2.P.5.6 Justification of Specification.

Given the fact that IVE data deviates significantly from the normal distribution, two sets of statistical analyses were performed: 1. Nonparametric tolerance interval that does not depend on any assumption of the data distribution (i.e. accept the data distribution as is); 2.[redacted] based on the logit transformation of the IVE data.

The nonparametric tolerance interval analysis shows that at least [redacted] of the population will be above the minimum value [redacted] with confidence of [redacted] if the same performance is carried forward to the future.

The second set of analysis is based on the normal distribution of the logit transformed data. Let p be the measured %positive values, then the corresponding logit transformation of p is

defined as

$$log_{10}\left(\frac{p}{100-p}\right)$$

The logit transformed data is much closer to the normal distribution as indicated in the histogram and the Q-Q plot below (for the logit transformed scale, the histogram is more symmetric and the data are more aligned around the straight line in the Q-Q plot) (Figure 3.2.P.5.6-4). The [redacted] based on the logit transformed data is [redacted], which corresponds to [redacted] in the original scale.

Figure 3.2.P.5.6-4. Histogram and Q-Q Plots of IVE Data of Original and Logit Transformed Scales

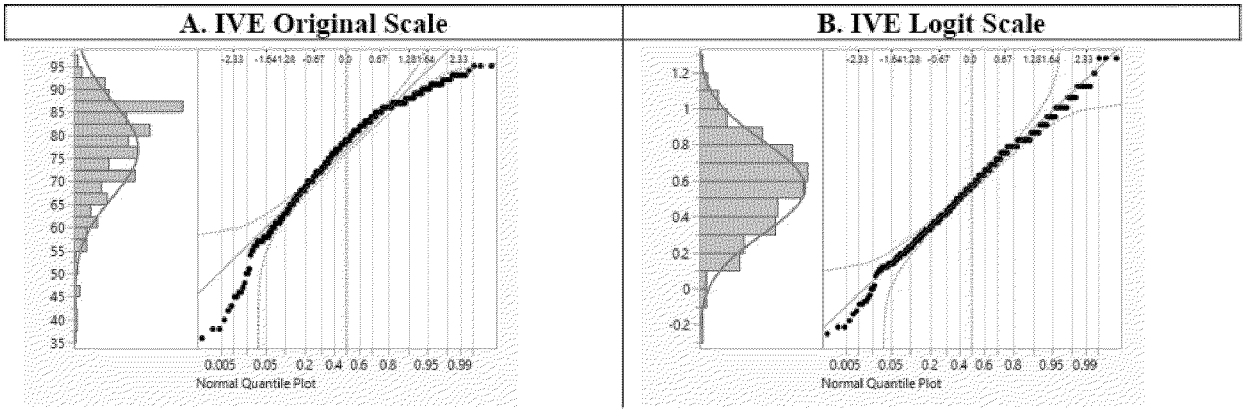


Table 3.2.P.5.6-24. BNT162b2 Drug Product In Vitro Expression (%) Data Summary: Statistical Analysis

	Analysis, N=654
Mean	[redacted]
SD	
Min, Max	
Mean-3SD[a]	

a. Calculated via logit transformation, and then converted back to the original scale

The analysis from nonparametric tolerance interval (min is [redacted]) and [redacted] based on the logit transformation of IVE data are very similar. With a large release data of N=654, the min value from nonparametric tolerance interval, [redacted] is considered appropriate as the acceptance criterion (Table 3.2.P.5.6-25). Stability data generated after the implementation of improved method at recommended storage were within the range of [redacted] (3.2.P.8.3 Stability Data - Long-Term). The proposed updated acceptance criterion is [redacted] S1+ cells for release and stability.

IVE specification is revised in 3.2.P.5.1 Specification.

Assessment of the MAH's response

The MAH has now provided data from 654 batches released after implementation of the method improvement. Of these 654 batches, 3 batches (0.45%) were released with values of [redacted] and additional 6 batches (0.91%) were released with potency values between [redacted] The company states that since IVE data deviates from the normal distribution, two sets of statistical analyses were performed to support the proposed revised limit. Based on these analyses, the specification for the in vitro expression assay is proposed to be revised to [redacted] S1+ cells for release and stability. However, clinical justification has not been taken into consideration in the justification of specification. From a clinical qualification perspective and taking in consideration the low frequency of batches with potency under [redacted], the proposed limit of [redacted] is not considered acceptably justified. The proposed limit should be further justified based on batches used in clinical trials or based on additional clinical knowledge (e.g from dose finding studies). This is considered especially important as the cell based in vitro expression assay is also an indirect control of the cellular uptake.

Conclusion

Issue remains

- The proposed limit for the in vitro expression test should be further justified based on release data from batches used in clinical trials or based on relevant clinical knowledge (e.g regarding dose/potency needed for an acceptable immune response). Module 3.2.P.5.6 of the dossier should be updated with this justification. (From SO2a, SO2e and REC10)

Question 8

Since only 3 months stability data are provided at long-term conditions for DP batches manufactured at Polymun (EM4965 and EL7834), the 6 months data are expected to be submitted in a future variation. (REC20)

Summary of the MAH's response

The 6-month stability data for DP lots EM4965 and EL7834 is updated in 3.2.P.8.3 Stability – Long Term. These updates have been made using the current approved 3.2.P.8.3 Stability – Long Term (sequence 00214).

3.2.P.8.1 Stability Summary and Conclusion has also been updated to reflect the data presented in 3.2.P.8.3 Stability – Long Term.

Assessment of the MAH's response

The requested 6 months stability data for the DP batches EM4965 and EL7834 manufactured at Polymun have been provided and included in section 3.2.P.8.3.

This is found acceptable.

Conclusion

Issue resolved.

10. Second request for supplementary information

10.1. Major objections

None.

10.2. Other concerns

Quality aspects

1. The proposed limit for the in vitro expression test should be further justified based on release data from batches used in clinical trials or based on relevant clinical knowledge (e.g regarding dose/potency needed for an acceptable immune response). Module 3.2.P.5.6 of the dossier should be updated with this justification. (From SO2a)

☐Overall conclusion and impact on benefit-risk balance has been updated accordingly

☐No need to update overall conclusion and impact on benefit-risk balance

11. Specific obligations and Recommendations

All specific obligations and recommendations included in this variation are considered fulfilled.

12. Assessment of the responses to the request for supplementary information

12.1. Major objections

Quality aspects

none

12.2. Other concerns

Quality aspects

Question 1
The proposed limit for the in vitro expression test should be further justified based on release data from batches used in clinical trials or based on relevant clinical knowledge (e.g regarding dose/potency needed for an acceptable immune response). Module 3.2.P.5.6 of the dossier should be updated with this justification. (From SO2a)

Summary of the MAH's response
The Marketing Authorization Holder proposes to tighten the acceptance criterion for the in vitro expression (IVE) test from "[redacted]Cells Positive" to "[redacted]Cells Positive" based on assessment of data collected from drug product lots used in initial clinical studies, conducted to establish safety and efficacy of the vaccine, commercial / emergency supply lots subsequently used in additional clinical studies, as well as lots used for emergency supply (so-called "Israel Lots"), where published real-world evidence of efficacy is available. The results of the IVE test obtained for these drug product lots are provided in Table 1. The proposed acceptance criterion is set to the lowest test result obtained for clinical drug product lots used to establish the safety and efficacy of the BNT162b2 vaccine.

During clinical development, the in vitro expression assay was used to characterize spike protein expression prior to its implementation as a drug product release test. Clinical drug product lots were tested at different RNA input amounts, and ultimately [redacted] which is the IVE assay condition currently used for product release. Because the final IVE assay conditions were implemented later in development, only clinical lots which were tested with [redacted] input are considered here. The spike protein expression results presented in Table 1 for lots BCV40720-A, BCV40720-B, BCV40720-C, ED3938, and EE3813 were collected at the time of clinical drug product lot release using the 150 ng level. The range of spike protein expression for these lots is [redacted] The spike protein expression results collected for

clinical lots during comparability studies, also tested at the [redacted] level, were previously presented, and are tabulated alongside results obtained at the time of lot release for clinical lots BCV40720-A, ED3938 and EE3813. Where two results are available, the spike protein expression results within each lot are in good agreement and differences are indicative of variability associated with this cell-based assay.

Table 1. BNT162b2 Clinical Drug Product Lots and Spike Protein Expression Results

Drug Product Lot Number	Drug Substance Batch Number	Purpose of Material	In Vitro Expression at Release (% cells positive)	In Vitro Expression in Comparability (% cells positive) [a]
BCV40420-A	R427-P020.2-DS	Clinical, Stability	Not Tested at [redacted] ng [b]	[redacted]
BCV40620-A	R438-P020.2-DS	Clinical, Stability	Not Tested at [redacted] ng [b]	
BCV40620-B		Clinical	Not Tested at [redacted] ng [b]	Not Tested
BCV40620-C		Clinical	Not Tested at [redacted] ng [b]	Not Tested
BCV40620-D		Clinical	Not Tested at [redacted] ng [b]	[redacted]
BCV40720-A	R443-P020.2-DS	Clinical, Stability	[redacted]	
BCV40720-B		Clinical		Not Tested
BCV40720-C		Clinical, stability		Not Tested
ED3938 (BCV40720-P)		Clinical, Stability		[redacted]
EE3813 (BCV40820-P)	R445-P020.2-DS	Clinical, Stability		
EE8492	20Y513C101	Commercial Supply / Emergency Supply, Clinical, Stability		NA
EE8493	20Y513C101	Commercial Supply / Emergency Supply, Clinical, Stability		NA
EK4175	20E162002 (1071542)	Commercial Supply / Emergency Supply, Israel Lot		NA
EK4237	20E162005 (1071546)	Commercial Supply / Emergency Supply, Israel Lot		NA
EK4241	20E162003 (1071544)	Commercial Supply / Emergency Supply, Israel Lot		NA
EK4238	20E162004 (1071545)	Commercial Supply / Emergency Supply, Israel Lot		NA
EK4240	20E162007 (1071548)	Commercial Supply / Emergency Supply, Israel Lot		NA
EL0200	20E162008 (1071551)	Commercial Supply / Emergency Supply, Israel Lot		NA

Table 1. BNT162b2 Clinical Drug Product Lots and Spike Protein Expression Results

Drug Product Lot Number	Drug Substance Batch Number	Purpose of Material	In Vitro Expression at Release (% cells positive)	In Vitro Expression in Comparability (% cells positive) [a]
EK4242	20E162007 (1071548)	Commercial Supply / Emergency Supply, Process performance qualification, Stability, Israel Lot	[redacted]	NA
EJ3002	20E162009 (1071552)	Commercial Supply / Emergency Supply, Israel Lot		NA
EL0203	20E162008 (1071551)	Commercial Supply / Emergency Supply, Israel Lot		NA
EL1404	20E162008 (1071551)	Commercial Supply / Emergency Supply, Israel Lot		NA
EJ0553	20Y513C501	Commercial Supply / Emergency Supply, Clinical, Stability		NA
EJ1685	20E162001 (1071539)	Commercial Supply/ Emergency Supply, Clinical, Stability		NA
EL1491	20E162001 (1071539)	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Clinical, Stability		NA
EL3248	20Y513C501	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Stability		NA
EL3249	20Y513C601	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Stability		NA
EL8723	20Y513C801	Commercial Supply / Emergency Supply, Clinical, Process performance qualification, Stability		NA
EM4965	20E162016 (1071559), 20E162017 (1071560)	Commercial Supply / Emergency Supply, Process performance qualification, Clinical, Stability		NA
ER9449	20E162018 (1071561)	Commercial Supply / Emergency Supply, Clinical		NA

Table 1. BNT162b2 Clinical Drug Product Lots and Spike Protein Expression Results

Drug Product Lot Number	Drug Substance Batch Number	Purpose of Material	In Vitro Expression at Release (% cells positive)	In Vitro Expression in Comparability (% cells positive) [a]
FC1526	(21E162041) 1077265, 21E162037 (1074771), 21E162043 (1077267)	Commercial Supply / Emergency Supply, Clinical	[redacted]	NA
FG8041	21E162057 (1077331)	Commercial Supply / Emergency Supply, Clinical, Stability		NA
FG3535	21Y513C7301	Commercial Supply / Emergency Supply, Clinical		NA

[a] Testing performed within a comparability assessment of clinical and commercial drug product.

[b] Analysis includes only test sample results at the 150 ng input. Previous testing was performed at slightly different assay conditions and although valid, are not considered in this assessment.

Assessment of the MAH's response

Additional data from batches used in the clinical trials have been provided with a range of spike protein expression between [redacted]. Consequently, the Marketing Authorization Holder proposes to tighten the acceptance criterion for the in vitro expression (IVE) test from "[redacted] Cells Positive" to "[redacted] Cells Positive". The approach is endorsed, and the newly proposed limit is considered clinically justified. Relevant modules of the dossier have been updated.

Issue solved.

Conclusion

☐Overall conclusion and impact on benefit-risk balance has been updated accordingly

☒No need to update overall conclusion and impact on benefit-risk balance

Specific obligation	Status
SO1: In order to complete the characterisation of the active substance and finished product, the MAH should provide additional data. **Due date: July 2021. Interim reports: March 2021.**	
a) Additional data is to be provided to further characterise the truncated and modified mRNA species present in the finished product both from process 1 and 2. Data are expected to cover batches used in clinical trials (for which the characterisation data could be available earlier) and the PPQ batches. These data should address results from ion pairing RP-HPLC addressing 5'cap levels and presence of A30 and L70 in the poly(A) tail. These data should further address the potential for translation into truncated S1S2	**Fulfilled (with a new recommendation)** SOB/003 monthly report (Jan21); MAH has confirmed that performance of the additional studies in relation to characterisation of active substance and control strategy for active substance and finished product have been started. No delay is encountered, so the interim reports stays on schedule. Monthly report (Feb21) eCTD seq 0041. No

Specific obligation	Status
proteins/peptides or other proteins/peptides. Relevant protein/peptide characterization data for predominant species should be provided. Any homology between translated proteins (other than the intended spike protein) and human proteins that may, due to molecular mimicry, potentially cause an autoimmune process should be evaluated. **Due date: July 2021. Interim reports: March 2021, and on a monthly basis.**	delays, still on track. SOB 003.1, interim report, concluded CHMP May 2021: A table summarising the activities initiated to fulfil SO1 is provided. Even though a more detailed interim report was expected, the table sufficiently confirms that work is in progress to address issues SO1a, b and c. The target completion date for all individual actions are stated and the final report for SO1 will be completed before the due date in July 2021. This is found acceptable. The rationale for the activity "Characterize 5'-cap levels and presence of A30 and L70 in the poly(A) tail" for SO1b is stated to be solely to demonstrate that the intact mRNA does include both 5'-Cap and poly(A) tail elements. The Applicant is reminded that the relative levels of 5'-Cap versus uncapped and poly(A) tail versus species without tail should be sufficiently reflected. SOB 003.3, concluded CHMP July 2021: The commitment to provide a monthly update (June 28) on specific obligation SO1a has been fulfilled. Further actions are required to fulfil the specific obligation SO1a including the submission of the required data by July 2021. VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: The Applicant has provided additional characterisation data. The potential for truncated transcripts to produce proteins/peptides was further investigated using a cell-free in vitro expression system. New Recommendation: The MAH should complement the characterization exercise using the cell-free in vitro translation system with additional tozinameran batches (at least three) representing the range of low to high RNA

Specific obligation	Status
	integrity levels at release.
b) The analysis of the main peak of the RNA integrity test representing the full-length RNA, should be also undertaken using the ion pairing RP-HPLC addressing 5'cap levels and presence of A30 and L70 in the poly (A) tail. **Due date: July 2021. Interim report: March 2021**	**Fulfilled** SOB 003.1, interim report, concluded CHMP May 2021: see above. SOB 003.2, CHMP conclusion June 2021: The commitment to provide a monthly update (May 25) on specific obligation SO1a has been fulfilled. Further actions are required to fulfil the specific obligation SO1a including the submission of the required data by July 2021. VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: It is sufficiently demonstrated that the major proportion of fragmented species contains the 5'-cap but lacks the poly(A) tail.
c) Additional data for the active substance are to be provided to confirm the identities of the observed Western Blot (WB) bands obtained by the *in vitro* expression assay. Protein heterogeneity, resulting in broad bands on the WB and uncertainties in the theoretical intact molecular weight of the spike protein, is assumed to be due to glycosylation. Therefore, to further confirm protein identities, enzymatic deglycosylation of the expressed proteins followed by WB analysis should be performed. Correlation with the calculated molecular weights of the intact S1S2 protein should be demonstrated. **Due date: July 2021. Interim report: March 2021**	**Fulfilled** SOB 003.1, interim report, concluded CHMP May 2021: see above. VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: WB results obtained by three different antibodies, specific for the S1 domain, the receptor binding domain and the S2 domain, respectively, are presented and compared to theoretical masses of the S-protein and the subdomains in glycosylated and non-glycosylated forms. It is sufficiently justified that the major band monitored corresponds to the heavily glycosylated S-protein.
SO2: In order to ensure consistent product quality, the MAH should provide additional information to enhance the control strategy, including the active substance and finished product specifications. **Due date: July 2021. Interim reports: March 2021.**	
a) The active substance and finished product specifications acceptance limits, being wider than the actual ranges for which clinical experience is	**Fulfilled** SOB/004 monthly report (Jan21); MAH has

Specific obligation	Status
available now, should be re-assessed and revised as appropriate, as further data becomes available from ongoing clinical trials and in line with manufacturing process capability and stability data of the product. Comprehensive data should be provided comprising batch analyses of a suitable number of commercial batches as well as analyses of batches that have been used in the (ongoing) clinical trials. **Due date: July 2021, Interim reports March 2021, and on a monthly basis.**	confirmed that performance of the additional studies in relation to characterisation of active substance and control strategy for active substance and finished product have been started. No delay is encountered, so the interim reports stays on schedule. Monthly report (Feb21) eCTD seq 0041. No delays, still on track. VAR II-17: It is noted that for one mibe batch (210206) the in vitro expression is at the lower end of the acceptance criteria ([redacted] cell positive). This is not representative for historical batches, where the in vitro expression for most batches are [redacted]. This needs to be taken into considerations when re-assessing specification acceptance limit to fulfil SO2 a. VAR IB-31-G: The DS specification limit for mRNA integrity at release and end-of-shelf-life has been revised from [redacted]. SOB 004.1, interim report, concluded CHMP May 2021: a. The Applicant states that release data from a comprehensive set of emergency supply/commercial drug substance batches and drug product lots have been acquired. It is confirmed that the reassessment of the drug substance and drug product specification acceptance limits will be completed by July 2021. No data is provided with the current submission. However, the information is considered sufficient for the interim report. SOB 004.2, concluded CHMP June 2021: The commitments to provide a monthly update (May 25) on specific obligation SO2a and SO2f has been fulfilled. Further actions are required to fulfil the specific obligation SO2a including the submission of the required data by July 2021. SOB 004.3, concluded CHMP July 2021: The commitments to provide a monthly

Specific obligation	Status
	update (June 28) on specific obligation SO2a and SO2f has been fulfilled. Further actions are required to fulfil the specific obligation SO2a including the submission of the required data by July 2021. VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: For drug substance the acceptance criterion for 5'-Cap has been tightened from [redacted]. For drug product the tightening of acceptance criteria for osmolality, LNP size, RNA encapsulation, RNA content and lipids content (ALC-0315, ALC-0159, DSPC, cholesterol) are sufficiently justified and found acceptable. For drug product the acceptance criterion for the *in vitro* expression (IVE) test has been tightened from [redacted] based on release data from batches used in clinical trials.
b) Poly(A) tail length is considered a critical attribute, which should be controlled on each batch, even though comparable results were obtained until now. An active substance specification to control poly(A) length should be introduced. A suitable method should be developed and appropriate acceptance criteria should be set. **Due date: July 2021, Interim reports: March 2021**	**Fulfilled** SOB 004.1, interim report, concluded CHMP May 2021: b. The Applicant confirms that a new method to evaluate poly(A) tail length in the active substance has been developed and is currently qualified. Method validation will be completed by July 2021. The method will be included in the DS specification and a corresponding specification will be proposed. This is found acceptable. VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: A new IP-RP-HPLC has been introduced and acceptably validated.
c) The poly(A) tail percentage is considered a critical attribute, but uncertainties remain on the suitability of the method. Additional data should	**Fulfilled** SOB 004.1, interim report, concluded CHMP

Specific obligation	Status
be provided to support the suitability of the method used for %poly(A) tail or an alternative suitable assay should be developed and introduced. The Applicant should evaluate any potential overestimation of poly(A) tail by the ddPCR method. The %poly(A) tail should be characterised following any future active substance process changes. **Due date: July 2021, Interim reports: March 2021**	May 2021: c. The Applicant presents data supporting the suitability of the ddPCR method, as being capable of detecting changes in the poly(A) tail content. It is expected that these results and the full assessment of the ddPCR method will be presented in further details in the final report by July 2021. The Applicant confirms that the ddPCR method will be used in combination with the new method described in point b, for drug substance release testing. This is acknowledged. VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: The accuracy of the ddPCR method is sufficiently addressed.
d) Since mRNA integrity and polydispersity are CQAs for the efficacy of the medicinal product, the finished product acceptance criteria for these parameters should be revised as further data becomes available from ongoing clinical trials and in line with manufacturing process capability. **Due date: July 2021, Interim reports: March 2021.**	**Fulfilled** VAR IB-31-G: The acceptance criteria for mRNA integrity at release is tightened from [redacted] to ensure that the shelf-life specification will be met as increasing the in-use shelf-life to 1 month at 2-8° C. The acceptance criteria should be re-evaluated taken into account available data from ongoing clinical trials and manufacturing process capability to fulfil SO2 d. SOB 004.1, interim report, concluded CHMP May 2021: d. In line with the response to SO2a, reassessment of the specification limits for mRNA integrity and polydispersity will be completed in July 2021 VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: The justifications for no further tightening of the DP specifications for RNA integrity and LNP polydispersity

Specific obligation	Status
	have been acceptably justified.
e) Additional data should be provided to support the suitability of the method used for potency determination or an alternative suitable assay for this purpose should be developed and introduced. Then the finished product acceptance criteria for potency should be revised accordingly. **Due date: July 2021, Interim reports: March 2021**	**Fulfilled** SOB 004.1, interim report, concluded CHMP May 2021: e. The method used to determine potency has been further improved to reduce inter-assay and intra-assay variability and to reduce data trending. A table summarising the on-going related activities is provided, and the final report is confirmed to be completed in July 2021. VAR II-56-G to fulfil SO1 (characterisation), SO2 (specifications) + related changes. 60D TT: Rap AR 20 Sep, BWP 4-6 Oct RSI/Opinion 14 Oct VAR II-56-G: Additional details have been provided to support the suitability of the IVE assay (cell-based flow cytometry) used for potency determination.

Recommendation	Status
Active substance	
10. The MAH should discuss the results and the assay suitability for the cell-based flow cytometry and the western blot method used for biological characterization of protein expression for the active substance.	**Fulfilled** VAR II-56-G: Additional details have been provided to support the suitability of the IVE assay (cell-based flow cytometry) used for potency determination.
Finished Product	
20. The applicant should provide the 6 months stability data for the finished product process performance qualification batches for assessment as soon as they are available.	**Fulfilled** 6 months stability data for the DP batches EM4965 and EL7834 manufactured at Polymun have been provided and included in section 3.2.P.8.3. REC20 is resolved
<u>New Recommendation (ref: SO1a):</u> 27. The MAH should complement the characterization exercise using the cell-free in vitro translation system with additional tozinameran batches (at least three) representing the range of low to high RNA integrity levels at release.	**Not Fulfilled**

Reminders to the MAH

1. The MAH is reminded to submit an eCTD closing sequence with the final documents provided by Eudralink during the procedure (including final PI translations, if applicable) within 15 days after the Commission Decision, if there will be one within 2 months from adoption of the CHMP Opinion, or prior to the next regulatory activity, whichever is first. If the Commission Decision will be adopted within 12 months from CHMP Opinion, the closing sequence should be submitted within 30 days after the Opinion or 5 days after the submission by the MAH of the final language translations, when there is a linguistic review. For additional guidance see chapter 4.1 of the Harmonised Technical Guidance for eCTD Submissions in the EU