

2.3.S.3. CHARACTERISATION

This section contains information specific for presentation Tris/sucrose Comirnaty [Original and Omicron (B.1.1.529)], which is discontinued. For information purposes, data/information supportive of the platform development approach for other presentations is maintained.

2.3.S.3.1. Elucidation of Structure and Other Characteristics

Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics describes the structure and characteristics of BNT162b2 drug substance (DS) which have been assessed using the analytical approaches outlined in Table 2.3.S.3-1. The analytical methodologies employed for BNT162b2 RNA drug substance characterization are capable of evaluating primary structure, including 5'-capping and 3'-poly(A) tail, and higher order structure. The results demonstrate that BNT162b2 RNA drug substance has the expected structure.

Analytical characterization was performed with BNT162b2 drug substance batch (20Y513C101).

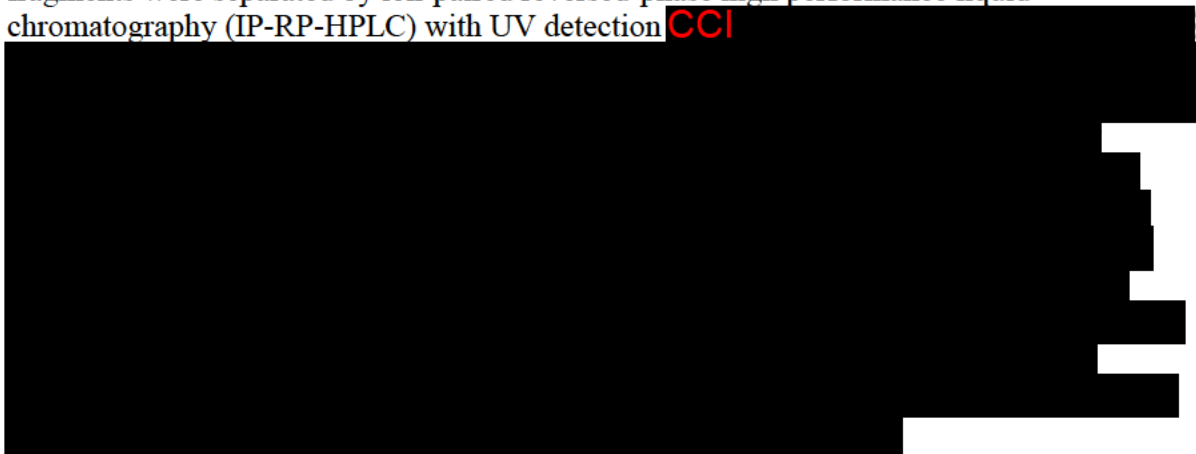
Table 2.3.S.3-1. Characterization Strategy for BNT162B2 Drug Substance

Characteristic	Analytical Approach	Methodology	Section References
Primary structure	Confirm expected RNA sequence at the oligonucleotide level	Reversed phase HPLC-UV and tandem mass spectrometry (LC/MS/MS) – of oligonucleotide fragments generated by RNase T1 digestion	Section 2.3.S.3.1.1.1
	Confirm the presence and determine the length of poly(A)-tail		Section 2.3.S.3.1.1.2
5'-Cap structure	Confirm the 5' capping structure and 5'-end profile	Reversed phase HPLC-UV and mass spectrometry (LC-UV/MS) analysis of purified 5' terminal after RNaseH digestion	Section 2.3.S.3.1.1.3
Poly(A)-tail	Confirm the presence and determine the length of poly(A)-tail	Reversed phase HPLC-UV and mass spectrometry (LC-UV/MS) analysis of purified poly(A)-tail after Ribonuclease T1 digestion	Section 2.3.S.3.1.1.4
Higher order structure (HOS)	Spectroscopic analysis to confirm the presence and fingerprint of HOS	Circular dichroism (CD) spectroscopy	Section 2.3.S.3.1.1.5
Biological Activity	Confirm size of expressed protein	Western blot analysis Cell-free <i>in vitro</i> translation	Section 2.3.S.3.1.1.6

2.3.S.3.1.1. Primary Structure

2.3.S.3.1.1.1. LC/MS/MS - Oligonucleotide Mapping

The primary sequence of BNT162b2 DS was analyzed by LC/MS/MS - oligonucleotide mapping. BNT162b2 DS was digested with RNase T1, and the resulting enzymatic fragments were separated by ion-paired reversed-phase high performance liquid chromatography (IP-RP-HPLC) with UV detection. CCI



CCI




The LC/MS/MS – oligonucleotide mapping results are summarized in Table 3.2.S.3.1-3 and demonstrate that BNT162b2 DS contains the correct sequence as predicted from the linear DNA template (Section 3.2.S.2.3 Control of Materials – Source, History and Generation of Plasmids).

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

2.3.S.3.1.1.2. Sequencing of RNA

In order to further confirm sequence identity, RNA sequencing for BNT162b2 DS was performed. CCI



Taken together, the RNA sequencing results further demonstrate that the BNT162b2 transcript generated during the *in vitro* transcription (IVT) process bears the correct RNA sequence as predicted from the linear DNA template.

2.3.S.3.1.1.3. 5'-Cap Characterization by LC-UV/MS

The characterization of the 5' end capped (5'-Cap) and un-capped species of BNT162b2 DS was accomplished by ion-pair reversed-phase high performance liquid chromatography-ultraviolet light detection (CCl) and online electrospray ionization mass spectrometry (IP-RP-HPLC/UV-ESI MS) or LC-UV/MS. Sample handling and chromatography follow the method described in [Section 3.2.S.4.2 Reversed Phase – High Performance Liquid Chromatography \(RP-HPLC\)](#).

CCl

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

Overall, characterization of BNT162b2 DS with the 5'-Cap method confirms that the

CCl

CCl with the 5'-Cap structure, CCl

2.3.S.3.1.1.4. 3' Poly(A)-tail Characterization by LC-UV/MS

Analysis of the 3' polyadenosine tail (poly(A)-tail) of BNT162b2 DS was accomplished by ion-pair reversed-phase high performance liquid chromatography with UV detection at (CCl) and on-line electrospray ionization mass spectrometry (RP-HPLC-UV/ESI MS or LC-UV/MS). The poly(A)-tail of BNT162b2 DS was cleaved off by ribonuclease T1 (RNase T1) followed by isolation via (CCl) affinity purification.

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics](#).

The (CCl) results demonstrate that BNT162b2 DS contains the expected poly(A)-tail A30 and L70 segments (CCl)

2.3.S.3.1.1.5. Higher Order Structure

The higher order structure of BNT162b2 mRNA DS was characterized in solution using

CCl

CCI

2.3.S.3.1.1.6. Biological Activity of BNT162B2 DS

To characterize the biological activity of BNT162b2 Omicron BA.1 (B.1.1.529) RNA DS, a Western blot was used to evaluate the size of the expressed protein through transfection into CCI. In addition, a cell-free *in vitro* translation method was used to detect the presence of a full-length of the expressed S1S2 protein.

2.3.S.3.2. Impurities

Data for this section is pending and will be updated once the data has been generated, analyzed, and verified.

This section summarizes the impurities that are controlled and monitored during the BNT162b2 drug substance manufacturing process.

Commercial scale batch testing results demonstrate that the process is capable of effectively and consistently delivering drug substance with acceptable levels of process and product related impurities and potential contaminants listed are listed in this section. Process validation data collected to date is provided for each impurity and will continue to be updated as testing for additional process validation batches is complete.

2.3.S.3.2.1. Process-Related Impurities

Process-related impurities are defined as impurities that originate from the manufacturing process and may be derived from reagents used in the in-vitro transcription and purification processes.

The process related impurities discussed in this section include residual DNA template.

2.3.S.3.2.2. Residual DNA Template

Residual DNA template is a process-related impurity derived from the linearized DNA template added to the in-vitro transcription reaction. Residual DNA template is further controlled through routine testing using the analytical procedure described in [3.2.S.4.2 Quantitative Polymerase Chain Reaction \(qPCR\)](#) and the BNT162b2 drug substance specification as described in [3.2.S.4.1 Specification](#).

2.3.S.3.2.3. Additional Process Related Impurities

The additional process related impurities that were evaluated include nucleoside triphosphates (NTPs) and capping structure, small molecules, and enzymes. For further information about the evaluation of potential process-related impurities and result refer to [Section 3.2.S.2.6 Risk Assessment of Potential Process Related Impurities](#).

2.3.S.3.2.2. Product-Related Impurities

The product related impurities discussed in this section include double stranded RNA. Safety assessment of double stranded RNA was performed as part of specification setting. Refer to [3.2.S.4.5 Justification of Specifications](#) for information pertaining to levels of these impurities relative to patient safety.

Double Stranded RNA

Double stranded RNA is a product-related impurity derived from the in-vitro transcription reaction. Double stranded RNA is further controlled through routine testing using the analytical procedure described in [3.2.S.4.2 Immunoblot](#) and the BNT162b2 drug substance specification as described in [3.2.S.4.1 Specification](#).

Potential Contaminants

Potential contaminants are defined as any adventitiously introduced materials (e.g., chemical, biochemical, or microbial species) not intended to be part of the manufacturing process of the drug substance or drug product (ICH Q6B).

The potential contaminants that may be present in BNT162b2 drug substance are endotoxin and bioburden.

During manufacture of the BNT162b2 drug substance, the manufacturing process has successfully been shown to effectively and consistently deliver drug substance with acceptable levels of the process and product related impurities and potential contaminants.