

## TABLE OF CONTENTS

LIST OF TABLES .....	1
2.3.S.3. CHARACTERISATION .....	2
2.3.S.3.1. Elucidation of Structure and Other Characteristics.....	2
2.3.S.3.1.1. Primary Structure.....	3
2.3.S.3.1.1.1. LC/MS/MS - Oligonucleotide Mapping .....	3
2.3.S.3.1.1.2. 5'-Cap Characterization by LC-UV/MS .....	3
2.3.S.3.1.1.3. 3' Poly(A)-tail Characterization by LC/MS/MS – Oligonucleotide Mapping .....	4
2.3.S.3.1.1.4. Higher Order Structure.....	4
2.3.S.3.1.1.5. Biological Activity ofOMICRON (BA.4/BA.5) DS.....	4
2.3.S.3.2. Impurities .....	5
2.3.S.3.2.1. Process-Related Impurities .....	5
2.3.S.3.2.2. Residual DNA Template .....	5
2.3.S.3.2.3. Additional Process Related Impurities .....	5

## LIST OF TABLES

Table 2.3.S.3-1. Characterization Strategy forOMICRON (BA.4/BA.5) Drug Substance .....	2
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### 2.3.S.3. CHARACTERISATION

This section contains information specific for presentation Comirnaty [Original and Omicron (BA.4/BA.5)], 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 [Omicron BA.4/BA.5) Variant] describes the structure and characteristics of Omicron (BA.4/BA.5) Omicron (BA.4/BA.5) drug substance (DS) which have been assessed using the analytical approaches outlined in Table 2.3.S.3-1. The analytical methodologies employed for Omicron (BA.4/BA.5) Omicron (BA.4/BA.5) RNA drug substance characterization are capable of evaluating p primary structure, including 5'-capping and 3'-poly(A) tail, higher order structure and biological activity. The results demonstrate that Omicron (BA.4/BA.5) Omicron (BA.4/BA.5) RNA drug substance has the expected structure.

Analytical characterization was performed with Omicron (BA.4/BA.5) drug substance confirmatory batch (GH5745).

**Table 2.3.S.3-1. Characterization Strategy for OMICRON (BA.4/BA.5) 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	<a href="#">Section 2.3.S.3.1.1.1</a>
	Confirm the presence and determine the length of poly(A)-tail		<a href="#">Section 2.3.S.3.1.1.3</a>
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	<a href="#">Section 2.3.S.3.1.1.2</a>
Higher order structure (HOS)	Spectroscopic analysis to confirm the presence and fingerprint of HOS	Circular dichroism (CD) spectroscopy	<a href="#">Section 2.3.S.3.1.1.4</a>
Biological Activity	Confirm size of expressed protein	Western blot analysis Cell-free <i>in vitro</i> translation	<a href="#">Section 2.3.S.3.1.1.5</a>

### 2.3.S.3.1.1. Primary Structure

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

The primary sequence of Omicron (BA.4/BA.5) DS was analyzed by LC/MS/MS - oligonucleotide mapping. Omicron (BA.4/BA.5) 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 at CCI [REDACTED]

CCI [REDACTED]

The LC/MS/MS – oligonucleotide mapping results are summarized in Table 3.2.S.3.1-3 and demonstrate that Omicron (BA.4/BA.5) 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 [Omicron BA.4/BA.5) Variant].

#### 2.3.S.3.1.1.2. 5'-Cap Characterization by LC-UV/MS

The characterization of the 5' end capped (5'-Cap) and un-capped species of Omicron (BA.4/BA.5) DS was accomplished by ion-pair reversed-phase high performance liquid chromatography-ultraviolet light detection at CCI [REDACTED] 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).

CCI [REDACTED]

CCI

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics \[Omicron BA.4/BA.5\) Variant\]](#).

CCI

#### **2.3.S.3.1.1.3. 3' Poly(A)-tail Characterization by LC/MS/MS – Oligonucleotide Mapping**

Heightened characterization of the 3' polyadenosine tail (poly(A)-tail) of Omicron (BA.4/BA.5) DS was accomplished CCI

Further details are provided in [Section 3.2.S.3.1 Elucidation of Structure and Other Characteristics \[Omicron BA.4/BA.5\) Variant\]](#).

The LC-UV/MS results demonstrate that Omicron (BA.4/BA.5) DS contains the expected poly(A)-tail A30 and L70 segments CCI

#### **2.3.S.3.1.1.4. Higher Order Structure**

The higher order structure of Omicron (BA.4/BA.5) mRNA DS was characterized in solution using circular dichroism (CD) spectroscopy. A CD spectrum is a measure of differential absorption of the left- and the right-circularly polarized light by the test article, which arises due to structural asymmetry. The ordered structure of mRNA yields a CD spectrum that may contain positive and/or negative signals, while the absence of a CD signal generally indicates a lack of ordered structure.

CCI

#### **2.3.S.3.1.1.5. Biological Activity ofOMICRON (BA.4/BA.5) DS**

To characterize the biological activity of Omicron (BA.4/BA.5) DS, a Western blot analysis was used to evaluate the size of the expressed protein using in vitro translation.

### **2.3.S.3.2. Impurities**

This section summarizes the impurities that are controlled and monitored during the 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 Omicron (BA.4/BA.5) drug substance specification as described in 3.2.S.4.1 Specification Omicron BA.4/BA.5.

#### **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 Omicron (BA.4/BA.5) drug substance specification as described in 3.2.S.4.1 Specification Omicron BA.4/BA.5.

### 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 Omicron (BA.4/BA.5) drug substance are endotoxin and bioburden.

During manufacture of the 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.