

2.3.S.4. CONTROL OF DRUG SUBSTANCE,OMICRON VARIANT

2.3.S.4.1. Specification

The specification for OmicronVariant drug substance at release and during stability studies is provided in Table 2.3.S.4-1. The acceptance criteria are applicable from batch release to end of shelf-life. The acceptance criteria provided are based on the available data. These criteria will be reassessed and amended as appropriate when more data become available.

Table 2.3.S.4-1. Omicron Omicron Drug Substance Specification

Quality Attribute	Analytical Procedure	Acceptance Criteria
Composition and Strength		
Clarity	Appearance (Clarity)	CCI
Coloration	Appearance (Coloration)	CCI
pH	Potentiometry	CCI
Content (RNA Concentration)	UV Spectroscopy	CCI
Identity		
Identity of Encoded RNA Sequence	ddPCR for Identity ^a	Identity confirmed
	RT-PCR for Identity ^{a,b}	
Purity		
RNA Integrity	Capillary Gel Electrophoresis	CCI
5'- Cap	RP-HPLC	CCI
Poly(A) Tail	ddPCR	CCI
Poly(A) Tail Length	IP-RP-HPLC	Poly(A) tail length confirmed
Process Related Impurities		
Residual DNA Template	qPCR ^a	CCI
Product Related Impurities		
dsRNA	Immunoblot ^a	CCI
Safety		
Bacterial Endotoxin	Endotoxin (LAL)	CCI
Bioburden	Bioburden	CCI

a. Assay not performed on stability.

b. Analytical Procedure used only at BioNTech Mainz

Abbreviations: NTU = nephelometric turbidity units; B = brown; RT-PCR = reverse transcription polymerase chain reaction; RP-HPLC = reverse phase high performance liquid chromatography; ddPCR = droplet digital PCR; IP-RP-HPLC = Ion-pair-reversed-phase high performance liquid chromatography; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = limulus amoebocyte lysate; EU = endotoxin unit; CFU = colony forming unit; PGS = Pfizer Global Supply; BNT =BioNTech

2.3.S.4.2. Analytical Procedures

Analytical procedures for Omicron variant drug substance (DS) release and stability testing are listed in Table 2.3.S.4-2. The test methods used for BNT162b2 Original DS (i.e. mRNA vaccine platform methods) are identical to those used for the variant DS, apart from the identity methods, which differ solely in the variant-specific PCR primers used in the assay.

Table 2.3.S.4-2. Analytical Procedures for Omicron Drug Substance

Analytical Procedure	Quality Attribute
Appearance	Clarity and Color
Potentiometry	pH
UV Spectroscopy	Content (RNA Concentration)
ddPCR for Identity	Identity of encoded RNA sequence
RT-qPCR for Identity	
RP-HPLC	5'-Cap
ddPCR	Poly (A) Tail
IP-RP-HPLC	Poly (A) Tail Length
qPCR	Residual DNA Template
Immunoblot	Residual dsRNA
Capillary Gel Electrophoresis	RNA Integrity
Endotoxin (LAL)	Bacterial Endotoxin
Bioburden	Bioburden

Abbreviations: ddPCR = digital droplet polymerase chain reaction, dsRNA = double stranded RNA, LAL = limulus amoebocyte lysate, qPCR = quantitative polymerase chain reaction, RP-HPLC = reversed phase-high performance liquid chromatography, IP-RPHPLC= Ion-pair-reversed-phase high performance liquid chromatography; RT-PCR = reverse transcription-polymerase chain reaction

2.3.S.4.3. Validation of Analytical Procedures

Validation or verification of analytical procedures for BNT162b2 Original drug substance (DS) was performed to ensure composition, strength, identity, purity, and safety.

Non-compendial analytical procedures were confirmed suitable for their intended use by assessing all relevant validation elements described in ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology. Compendial analytical procedures were verified or validated according to the appropriate USP, Ph. Eur., and JP chapters.

The DS concentration, formulation process, quality attributes, and process control remain unchanged in the variant. The test methods used for BNT162b2 Original DS (i.e. mRNA vaccine platform methods) are identical to those used for the variant DS, apart from the identity methods, which differ solely in the variant-specific PCR primers used in the assay. The supplemental validations for the identity methods, digital droplet polymerase chain reaction (ddPCR) and reverse transcription-polymerase chain reaction (RT-PCR), for the variant DS are referenced in [Section 3.2.S.4.3 Validation of Analytical Procedures – Overview \[Omicron \(JN.1\) Variant\]](#). The supplemental ddPCR validation was performed at a single laboratory (Pfizer Global Supply, Grange Castle) to confirm method performance. Upon completion of the supplemental validation, the other qualified and licensed laboratories may implement the ddPCR method for the current variant using the appropriate site quality systems.

The remainder of the DS analytical procedures are not impacted by the change to the mRNA sequence and do not require additional validation for the variant. Therefore, laboratories and test methods previously verified/validated for the testing of BNT162b2 Original DS are considered suitable for testing of the variant DS.

2.3.S.4.4. Batch Analyses

The information on Omicron (JN.1) drug substance (DS) batch manufactured using the same process as the BNT162b2 Original DS and used for clinical trials, process confirmatory studies, and stability studies summarized in [Section 3.2.S.4.4 Batch Analyses \[Omicron \(JN.1\) Variant\]](#). The batch met the release acceptance criteria in place at the time of release.

2.3.S.4.5. Justification of Specification

The specification for Omicron variant drug substance is based on an understanding of the control strategy and CQAs for BNT162b2 drug substance. For subsequent constructs (i.e. variant DS), the historical data and relevant assessments from the BNT162b2 drug substance are confirmed applicable, applied and unchanged, with the exception of identity. The attributes tested and associated acceptance criteria ensure the consistency of drug substance and linkage to clinical experience. This specification was established to ensure the quality, purity, potency/biological activity and safety of the commercial drug substance at release and during storage Specification-Setting Strategy.

Because there are no significant trends that would impact the shelf life of the drug substance

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the acceptance criteria used for stability over shelf life are the same as the acceptance criteria used for batch release. In summary, the acceptance criteria in the drug substance specification reflect the current understanding of criticality of quality attributes, their impact on product performance, their stability, and the quality of the product used in clinical trials.