3.2.S.2.6. ANALYTICAL METHOD EVOLUTION

This section contains information specific for formulations containing original drug substance, which are discontinued. For information purposes, data/information supportive of the platform development approach for other presentations is maintained.

The analytical testing strategy applied to BNT162b2 drug substance has evolved throughout the development history. These changes to the analytical testing strategy are summarized in Table 3.2.S.2.6-1.

Table 3.2.S.2.6-1. Evolution of BNT162b2 Drug Substance Methods

Process		Clinical (Process 1)	Emergency Supply[a] (Process 2)	Process Performance Qualification, Commercial Supply
Quality Attribute	**Analytical Procedure**	4.2 1st ind.		
Clarity	Appearance			
Coloration	Appearance			
pH	Potentiometry			
Osmolality	Osmometry			
RNA sequence	Sequencing of DNA starting material			
Content (RNA concentration)	UV Spectroscopy			
Identity: RNA length	Agarose gel electrophoresis			
Identity: as RNA				
Identity of encoded RNA sequence	RT-PCR			
RNA integrity	Capillary gel electrophoresis			
5'-Cap	HPLC-UV			
Poly(A) tail	ddPCR			
Residual DNA template	qPCR			
Residual double stranded RNA (dsRNA)	Immunoblot			
Bacterial endotoxins	Endotoxin (LAL)			
Bioburden	Bioburden			

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

4.2 1st ind. RT-PCR = reverse transcription polymerase chain reaction; ddPCR = droplet digital PCR; qPCR = quantitative PCR; dsRNA = double stranded RNA; LAL = Limulus amebocyte lysate

3.2.S.2.6.1. Description of Changes and Method Bridging

Description of method changes and results of appropriate bridging experiments are detailed in Sections 3.2.S.2.6.1.1 to 3.2.S.2.6.1.4. For some methods both the historic and new methods were tested concurrently, and the data compared.

3.2.S.2.6.1.1. Osmolality

4.2 1st ind. [redacted]

3.2.S.2.6.1.2. RNA Sequence by Sequencing of DNA Starting Material

Testing of the DNA starting material (process 1) to ensure the correct DNA template for transcription has been removed from the BNT162b2 drug substance release testing. With the change from DNA template to plasmid DNA, concomitant with the change from process 1 to process 2, the analytical control of the plasmid DNA sequence integrity occurs during plasmid release testing as described in Section 3.2.S.2.3. Source, History and Generation of Plasmids.

3.2.S.2.6.1.3. Agarose Gel Electrophoresis and RT-PCR

Agarose gel electrophoresis was originally positioned as an identity method for BNT162b2 drug substance. 4.2 1st ind. [redacted]

The agarose gel electrophoresis method has been replaced with the reverse transcription-polymerase chain reaction (RT-PCR) method to confirm the encoded RNA sequence, as described in Section 3.2.S.4.2 Analytical Procedures – RT-PCR. This method positively identifies the encoded RNA sequence as BNT162b2, and demonstrates identity as RNA, as only RNA can be reverse transcribed.

Specificity data generated during the qualification of the RT-PCR method, as presented in Section 3.2.S.4.2 Validation of Analytical Methods – RT-PCR, demonstrate that the method is suitable for use as an identity method for BNT162b2 drug substance. In addition, four batches of drug substance were analyzed by both the Agarose Gel Electrophoresis and RT-PCR methods. All batches passed the respective acceptance criteria for identity for both methods, as detailed in 3.2.S.2.6 Developmental History and Comparability Assessment.

3.2.S.2.6.1.4. Capillary Gel Electrophoresis

4.2 1st ind.

[redacted]

Table 3.2.S.2.6-2. Capillary Gel Electrophoresis - Comparison of Processing Methods

BNT162b2 Drug Substance Batch	RNA Integrity	RNA Integrity	RNA Integrity
R427-P020.2-DS	4.2 1st ind.		
R438-P020.2-DS			
R443-P020.2-DS			