Formulation to Filling Process Validation – Site

<INN> drug product process validation was conducted at Name of Site (AXX). The validation data demonstrated the process to be controlled, consistent, and reproducible.

The process was validated for X drug product presentations:

- <INN> XX mg (X.X mL) in PFS/Vial
- <INN> YY mg (X.X mL) in PFS/Vial

The <INN> drug product manufacturing activities at AXX included the following processing steps:

- Formulation buffer preparation
- Drug substance thaw
- Drug product formulation
- Bioburden reduction filtration
- Filtered formulated drug product hold and sterile filtration
- Aseptic filling and plunger-stopper/piston placement of the filled syringe/cartridges
- Lyophilization
- Capping

1. Establishment of Process Validation Strategy

The drug product process validation strategy was designed to demonstrate that the manufacturing process is controlled, reproducible, and consistently yields drug product having the required product quality attributes. The acceptable ranges for process parameters and the validation acceptance criteria for performance indicators were based on process understanding and prior knowledge gained from historical process development, process characterization, clinical manufacturing experience, and reference product data.

The number of validation lots was based on:

- comprehensive knowledge of product quality attributes and their criticality
- comprehensive understanding of the impact of the process on product quality attributes and a detailed understanding of potential sources of process variability and their control
- consistent and robust performance at clinical scale prior to process validation execution
- extensive operational experience in the commercial facility for similar processes

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2. Lots Used for Validation

Three consecutive lots were produced at AXX for each drug product presentation. Each validation lot was filled as a split lot with one formulation lot being used to fill two drug product lots, one for each presentation. The process validation batches were produced with a batch size range of XX.X kg to XX.X kg based on ± 10% of the filled minimum and maximum batch sizes of this campaign and taking into account equipment considerations. A summary of the validation lots is provided in table below.

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Table 1. Process Validation Summary

Presentation	Formulation Lot Number	Drug Product Lot Number	Drug Product Lot Date of Manufacture	Batch Size (kg)	Units Filled	Drug Substance Lot Number
XX mg	XXXXXXXXXX	XXXXXXXXXX	MMM YYYY	XX		
YY mg		XXXXXXXXXX	MMM YYYY			
XX mg	XXXXXXXXXX	XXXXXXXXXX	MMM YYYY	XX		
YY mg		XXXXXXXXXX	MMM YYYY			
XX mg	XXXXXXXXXX	XXXXXXXXXX	MMM YYYY	XX		
YY mg		XXXXXXXXXX	MMM YYYY			

3. Formulation Buffer Preparation

Drug product manufacturing starts with preparation of the formulation buffer (XX mM acetate and X% (w/v) sucrose adjusted to a pH of XX with XX mM sodium hydroxide). After buffer preparation, samples are collected prior to filtration for testing. The formulation buffer is filtered through a 0.X μm filter into a stainless-steel vessel at the point of use. All process parameters were within the acceptable ranges and all performance indicators were within the validation acceptance criteria as shown in the tables below.

Table 2. Formulation Buffer Preparation Process Parameter Results

Process Parameter	Acceptable Range	Formulation Lot Number		
		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Buffer vessel mixing speed (rpm)	XXX to XXX	XXX	XXX	XXX
Buffer vessel mixing time (min)	XX to XX	XX	XX	XX

Table 3. Formulation Buffer Preparation Performance Indicator Results

Performance Indicator	Validation Acceptance Criteria	Formulation Lot Number		
		XXXXXXXX	XXXXXXXX	XXXXXXXX
pH	X.X to X.X			
Osmolality (mOsm/kg)	XXX to XXX			
Conductivity (μS/cm)	XXX to XXX			
Bioburden (CFU/ 100 mL)	≤ XX			
Bacterial Endotoxins (EU/mL)	≤ X.XX			

CFU = colony forming units

4. Drug Substance Thaw and Formulation

Drug substance static thaw and formulation process steps were validated. After completion of the thaw and formulation, samples were collected from the formulated drug product prior to filtration into the storage vessel. All process parameters were within the acceptable ranges and all performance indicators were within the validation acceptance criteria as shown in the tables below.

Table 4. Thawing and Formulation Process Parameter Results

Process Parameter	Acceptable Range	Formulation Lot Number		
		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Thaw temperature (°C)	XX to XX	XX	XX	XX
Thaw time (hr)	≤ XX	XX	XX	XX
Formulation vessel mixing speed (rpm)	XXX to XXX	XXX	XXX	XXX
Formulation vessel mixing time (min)	XX to XX	XX	XX	XX

Table 5. Thawing and Formulation Performance Indicator Results

Performance Indicator	Validation Acceptance Criteria	Formulation Lot Number		
		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
pH	X.X to X.X	X.X	X.X	X.X
Osmolality (mOsm/kg)	XXX to XXX	XXX	XXX	XXX
Protein concentration (mg/mL)	X.X to X.X	X.X	X.X	X.X
Bacterial endotoxins (EU/mL)	≤ X.XX	X.XX	X.XX	X.XX
Polysorbate 80 (% w/v)	X.XXX to X.XXX	X.XXX	X.XXX	X.XXX

5. Bioburden Reduction Filtration

The formulated drug product is filtered through a 0.X μm filter for bioburden reduction into the storage vessel. All process parameters were within the acceptable ranges and all performance indicators were within the validation acceptance criteria as shown in the tables below.

Table 6. Bioburden Reduction Filtration Process Parameter Results

Process Parameter	Acceptable Range	Formulation Lot Number		
		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Filtration pressure (psig)	X to X	X	X	X

Table 7. Bioburden Reduction Filtration Performance Indicator Results

Performance Indicator	Validation Acceptance Criteria	Formulation Lot Number		
		XXXXXXXX	XXXXXXXXX	XXXXXXXXX
Pre-filtration Bioburden (CFU/10 mL)	≤ XX	X	X	X
Post-filtration filter integrity test (psig)[a]	Pass (≥ XX)	Pass (XX)	Pass (XX)	Pass (XX)

[a] Water for injection (WFI) is used as wetting agent to perform the test per established operating procedures

6. Filtered Formulated Drug Product Hold

The filtered formulated drug product was held at X°C to X°C during storage. The hold time in the storage vessel has been validated through the following:

- Completion of X consecutive media challenges supporting an aseptic hold duration of at least XXX hours in the XXX L storage vessel
- Establishment of chemical stability through XXX hours

Table 8. Media Challenge Hold Times

Process Parameter	Media Challenge Run		
	Run 1	Run 2	Run 3
Media hold time (hr)	XXX	XXX	XXX

Table 9. Media Challenge Results

Hold Interval	Performance Indicator	Validation Acceptance Criteria	Media Challenge Run		
			Run 1	Run 2	Run 3
Beginning	Bioburden	< 10 CFU/100 mL	X	X	X
	Growth promotion	Pass	Pass	Pass	Pass
End	Bioburden	< 10 CFU/100 mL	X	X	X
	Growth promotion	Pass	Pass	Pass	Pass

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Table 10. Filtered Formulated Drug Product Performance Indicator Results

Performance Indicator	Validation Acceptance Criteria	Hold Interval	Formulation Lot Number XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
SE-UHPLC					
Main peak (%)	≥ X.X	Beginning End			
HMW (%)	≤ X.X	Beginning End			
CEX-HPLC					
Acidic peaks (%)	XX.X to XX.X	Beginning End			
Main peak (%)	XX.X to XX.X	Beginning End			
Basic peaks (%)	XX.X to XX.X	Beginning End			
Polysorbate 80 (%w/v)	XXX.X to XXX.X	Beginning End			
pH	X.X to X.X	Beginning End			
Osmolality (mOsm/kg)	XXX to XXX	Beginning End			
Protein concentration (mg/mL)	X.X to X.X	Beginning End			
Bioburden (CFU/10 mL)	≤ X	Beginning End			
Bacterial endotoxins (EU/mL)	≤ XX	Beginning End			

7. Sterile Filtration

The drug product is sterilized through a 0.X μm filter. All process parameters were within the acceptable ranges and all performance indicators were within the validation acceptance criteria as shown in the tables below.

Table 11. Sterile Filtration Process Parameter Results

Process Parameter	Acceptance Criteria	Drug Product Lot Number		
		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Pressure (psig)	X to X			
Filling time (hr)[a]	≤ XX	XX.XX	XX.XX	XX.XX

[a] Acceptance criterion is the shortest allowed duration across aseptic process media fill validation, filter validation, and product hold durations. The shortest allowed duration for <INN> is the media fill validation time.

Table 12. Sterile Filtration Performance Indicator Results

Performance Indicator	Validation Acceptance Criteria	Drug Product Lot Number		
		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Pre-filtration Bioburden (CFU/10 mL)	≤ X	X	X	X
Post-filtration filter integrity test (psig)[a]	Pass (≥ XX)	Pass (XX)	Pass (XX)	Pass (XX)

[a] Water for injection (WFI) is used as wetting agent to perform the test per established operating procedures

8. Filling Validation

Filling validation demonstrated that the filling process is controlled, consistent, and reproducible. Fill weight control capability was demonstrated by periodic fill weight checks during the filling process. Product homogeneity across the filling operation was demonstrated by evaluating protein concentration, aggregation by SE-UHPLC, deamidation by CEX-HPLC, and subvisible particles by HIAC.

8.1 Filling Line Set Up Confirmation

Validation testing demonstrated that the protein concentration and polysorbate 80 concentration are not impacted by the adsorption to the sterile filter membrane and do not become diluted due to sterile condensate in the line that may reside in the filling equipment after sterilization. All performance indicators were within the validation acceptance criteria as shown in the tables below.

Table 13. Confirmation of Protein Concentration During Fill-line Set-up

Performance Indicator	Validation Acceptance Criteria	Vial/Syringe	Drug Product Lot Number		
			XXXXXXXXX	XXXXXXXXX	XXXXXXXXX
Protein concentration (mg/mL)	XX to XX	2			
		4			
		6			
		8			
		10			

Table 14. Confirmation of Polysorbate 80 During Fill-line Set-up

Performance Indicator	Validation Acceptance Criteria	Vial/Syringe	Drug Product Lot Number		
			XXXXXXXXX	XXXXXXXXX	XXXXXXXXX
Polysorbate 80 (% w/v)	X.XXX to X.XXX	1			
		3			
		5			
		7			
		9			

8.2 Fill Weight Evaluation

The consistency of fill weights was verified by automated fill weight data checks from all X validation runs. The data demonstrate that the filling process will consistently and reliably meet the acceptance criteria. The fill weight validation data are provided in the table below.

Table 15. Fill Weight Summary

Performance Indicator	Validation Acceptance Criteria		Drug Product Lot Number		
			XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
Fill weight (g)	X.XXX to X.XXX[a]	Minimum			
	Target: X.XXX	Maximum			
		Mean			
Process capability	≥ X.X	Ppk			
Number of data points	XXX				

[a] Equivalent to X.XX to X.XX mL volume when corrected for density (X.XXX g/mL) and subtracting hold-up volume (X.XXX mL).

8.3 Filling Process Homogeneity

Filling homogeneity was demonstrated for <INN> by evaluating the batch that yielded the largest number of filled units. The justification for the product quality attributes selected for testing is shown in the table below.

Table 16. Filling Homogeneity Quality Attributes

Quality Attribute	Justification	Analytical Method
Quantity	Demonstrates that the drug product content is maintained throughout the filling process	Protein concentration
Purity	Demonstrates the product quality is not adversely impacted over the duration of the fill through aggregation and / or oxidation	SE-UHPLC CEX-HPLC
Subvisible Particles	Quantitatively determines that subvisible particle content is maintained within specification throughout the filling process	Light obscuration

8.3.1 Filling Homogeneity Acceptance Criteria

The batch evaluated for filling homogeneity was equally divided into 3 sampling intervals. These intervals were labeled as “Beginning (B)”, “Middle (M)”, and “End (E)”.

Fill homogeneity by protein concentration, SE-UHPLC, and CEX-HPLC was tested via an average equivalence test. Homogeneity within the batch was demonstrated when the 2 one-sided 95% confidence intervals of the average differences between sampling intervals (eg, M vs B, E vs M, and E vs B) fell within the pre-defined equivalence acceptance criteria (EAC). Homogeneity for subvisible particulates was demonstrated when the results from all the sample locations were shown to have met the specification limits.

8.3.2 Filling Homogeneity Results

All homogeneity samples collected from filling met product specification and filling homogeneity and acceptance criteria shown in the tables below.

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Table 17. Filling Results Summary

Performance Indicator	Validation Acceptance Criteria	Interval	Lot XXXXXXXXX	
			Min	Max
Protein concentration (mg/mL)	X.X to X.X	Beginning Middle End		
Polysorbate 80 concentration (% w/v)	X.XXX to X.XXX	Beginning Middle End		
SE-UHPLC				
Main peak (%)	≥ X.X	Beginning Middle End		
HMW (%)	≤ X.X	Beginning Middle End		
CEX-HPLC				
Acidic peaks (%)	≥ X.X	Beginning Middle End		
Main peak (%)	≤ X.X	Beginning Middle End		
Basic peaks (%)	≤ X.X	Beginning Middle End		
Subvisible particles ≥ XX μm (particles per container)	≤ X	Beginning Middle End		
Subvisible particles ≥ XX μm (particles per container)	≤ X	Beginning Middle End		

Table 18. Statistical Evaluation of Homogeneity Data

Performance Indicator	Homogeneity Acceptance Criteria	Comparison	Mean Difference	95% Confidence Interval of Mean Difference		Conclusion
				Lower	Upper	
Protein concentration (mg/mL)	± X.XX	B ↔ M				Pass / Fail
		B ↔ E				Pass / Fail
		M ↔ E				Pass / Fail
Polysorbate 80 concentration (% w/v)	± X.XXX	B ↔ M				Pass / Fail
		B ↔ E				Pass / Fail
		M ↔ E				Pass / Fail
SE-UHPLC						
Main peak (%)	± X.XX	B ↔ M				Pass / Fail
		B ↔ E				Pass / Fail
		M ↔ E				Pass / Fail
HMW (%)	± X.XX	B ↔ M				Pass / Fail
		B ↔ E				Pass / Fail
		M ↔ E				Pass / Fail

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Table 19. Statistical Evaluation of Homogeneity Data

Performance Indicator	Homogeneity Acceptance Criteria	Comparison	Mean Difference	95% Confidence Interval of Mean Difference		Conclusion
				Lower	Upper	
CEX-HPLC						
Acidic peaks (%)	± X.XX	B ↔ M				Pass / Fail
		B ↔ E				Pass / Fail
		M ↔ E				Pass / Fail
Main peak (%)	± X.XX	B ↔ M				Pass / Fail
		B ↔ E				Pass / Fail
		M ↔ E				Pass / Fail
Basic peaks (%)	± X.XX	B ↔ M				Pass / Fail
		B ↔ E				Pass / Fail
		M ↔ E				Pass / Fail

9. Lyophilizer Performance

After all the filled vials were loaded into the lyophilizer, the vials underwent a freezing phase, followed by primary and secondary drying phases. The performance of the lyophilizer was checked with regard to shelf temperature and chamber vacuum for each phase of the cycle. The vials were closed by compressing the stoppers to full insertion within the vials and then capped. The lyophilization performance was checked at the end of each step with regard to shelf temperature and chamber pressure.

9.1 Consistency of the Lyophilization Process

At the end of the lyophilization cycle, samples were drawn from X locations on each shelf to test residual moisture. For reconstitution time, sampling was performed from X different locations on each shelf. The vials were stoppered completely and crimped manually. All results of residual moisture and reconstitution time met the acceptance criteria as shown in the table below.

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Table 19. Consistency of the Lyophilization Process

Performance Indicator	Validation Acceptance Criteria	Lot XXXXXXXXXX			Lot XXXXXXXXXX			Lot XXXXXXXXXX		
		No. of Samples	Min	Max	No. of Samples	Min	Max	No. of Samples	Min	Max
Residual moisture (%)	≤ X									
Reconstitution time (min)	< X									

9.2 Lyophilizer Homogeneity

Lyophilization process homogeneity was demonstrated with X process validation runs for drug product at maximum, minimum, and intermediate lot sizes.

Table 20. Lyophilization Homogeneity General Information

Drug Product Lot Number	Lot Size	Lyo Unit Used	Number of Full/Partial Shelves	Number of Units Filled
XXXXXXXXXX	Minimum	Lyo 1	X / X	XXX
XXXXXXXXXX	Maximum	Lyo 2	X / X	XXX
XXXXXXXXXX	Intermediate	Lyo 2	X / X	XXX

Samples were taken from multiple shelves and from up to X locations on each shelf. The actual number of samples taken from each shelf/location was determined individually for each quality attribute to obtain a targeted statistical power of 90% to discern the homogeneity hypothesis at the 95% confidence level. The justification for the product quality attributes selected for lyophilizer homogeneity testing is shown in table below.

Table 21. Lyophilizer Homogeneity Quality Attributes

Quality Attribute	Justification	Analytical Method
Quantity	Demonstrates that the drug product content is maintained throughout the filling process	Protein content
Purity	Demonstrates the product quality is not adversely impacted over the duration of the fill through aggregation and / or oxidation	SE-UHPLC CEX-HPLC
Moisture	Quantitatively determines that moisture is consistent throughout the lyophilizer	Residual moisture

Samples were collected for protein content, SE-UHPLC, CEX HPLC, and residual moisture. The sample results were statistically analyzed for homogeneity based on the prescribed statistical model.

The multi-regression model was used to estimate the minimum (low) and maximum (high) analyte averages within each lot. To test for analyte homogeneity, the 90% confidence interval (CI) of the maximum difference within a lot was then compared to pre-defined homogeneity acceptance criteria. As presented in table below, homogeneity was demonstrated for each validation lot, as the test attributes' 90% confidence interval of the maximum differences fell within the homogeneity acceptance criteria.

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The multi-regression statistical analysis for each validation lot demonstrates that the formulation, filling, and lyophilization process yields homogeneous drug product quality. The results also confirm the suitability of the current lot release sampling scheme by demonstrating that a sample taken from any point within the lyophilization chamber is representative of the lot.

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Table 22. Lyophilization Homogeneity

Performance Indicator	Number of samples	Equivalence Acceptance Criteria (90% CI Difference Criteria)	Drug Product Lot Number			Conclusion
			XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	
SE-UHPLC	XX					
Main peak (%)		± X.XX				Pass / Fail
HMW (%)		± X.XX				Pass / Fail
CEX-HPLC	XX					
Acidic peaks (%)		± X.XX				Pass / Fail
Main peak (%)		± X.XX				Pass / Fail
Basic peaks (%)		± X.XX				Pass / Fail
Moisture (%)	X	± X.XX				Pass / Fail
Protein content (µg/vial)	X	± X.XX				Pass / Fail

10. Capping Process

The capping process qualification of the drug product process validation was monitored during the production of the validation lots. The quality attributes checked after capping of the vials demonstrated that the capping operation was in a state of control.

11. Syringe Plunger-Stopper/Piston Placement

Placement of the plunger-stopper was qualified by measuring the plunger-stopper position in prefilled syringe samples collected from beginning, middle, and end stages of the filling and plunger-stopper placement process from all validation lots. All results met the acceptance criteria demonstrating that the plunger-stopper placement process is consistent and reproducible as shown in the table below.

Table 23. <INN> Syringe Plunger Stopper Summary

Parameter	Acceptance Criteria		Drug Product Lot Number		
			XXXXXXXXX	XXXXXXXX	XXXXXXX
Plunger depth (mm)	X.XX to X.XX	Minimum			
		Maximum			
		Mean			
Process capability	≥ X.XX	Ppk			
Number of data points	XXX				

12. Drug Product Release Specification Testing

All lot release results pass the proposed commercial specification. The lot release testing results for these lots are provided in 3.2.P.5.4 (Batch Analyses).

13. Conclusion

All validation runs met the acceptance criteria, demonstrating consistency and reliability of the manufacturing process at AXX. In conclusion:

- Process validation results were evaluated against predefined acceptance criteria. The results obtained during process validation demonstrate that drug product manufacturing process is validated and consistently meets criteria for process performance and product quality.
- Hold time data demonstrated that a filtered formulated drug product hold time of XXX hours is supported.
- Protein concentration and polysorbate 80 results demonstrated that the product is not affected by any condensation that may reside in the filling equipment or adsorption to the sterile filter membrane.

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- Fill weight check data demonstrated that the filling process consistently and reliably meets the release specification criteria and meets the process capability criterion.
- The statistical analysis of results of product quality attributes during filling demonstrated that there was no significant difference between samples taken throughout the lot, thus confirming homogeneity during filling.
- The plunger-stopper placement qualification results demonstrated that the plunger-stopper placement process is consistent and reproducible.
- Consistency and reliability of the prefilled syringe manufacturing process at AXX demonstrated that the manufacturing process is in a state of control.