1. Reference Standards

During the course of development, thoroughly characterized reference standards have been established to support INN manufacturing and testing. Early in development, the first INN reference standard (Lot XXXXXXXXXX) was prepared by the CP-X.X process as described in 3.2.S.2.6 (Process Development History). Later, a primary stability batch of INN (Lot XXXXXXXXXX) was qualified as the primary reference standard. This batch was manufactured using the CP-X.X. This process is equivalent to the proposed commercial manufacturing process CP-X.X described in 3.2.S.2.2 (Description of Manufacturing Process and Process Controls), as shown in 3.2.S.2.6 (Product Comparability). Lot XXXXXXXXXX, manufactured using the CP-X.X process, was qualified as the working (secondary) reference standard against the primary reference standard.

A program is in place to periodically requalify the primary reference standard, working (secondary) reference standard, achiral resolution standard, and chiral resolution standard.

2. Primary Reference Standard

2.1 Primary Reference Standard Preparation

The INN batch used to prepare the primary reference standard was manufactured by the initial CP-X.X process as described in 3.2.S.2.6 (Manufacturing Process Development, History), and released as Lot XXXXXXXXXX. A comparability analysis determined that the initial CP-X.X process produced drug substance of comparable quality to commercial batches discussed in 3.2.S.2.6 (Manufacturing Process Development, Product Comparability).

2.2 Primary Reference Standard Analysis

The physical and chemical properties of the primary reference standard are provided in 3.2.S.3.1 (Elucidation of Structure and Other Characteristics).

The results of additional testing performed for initial qualification of this reference standard are provided in Table 1.

The calculation of INN content in the primary reference standard was determind by applying the mass balance principle and assigned a purity using the following formula:

$$\%\ Purity = 100\ - \%TI - \%RS - \%Water - \%ROI$$

where:

% TI = total impurities obtained from HPLC method, expressed in w/w%

% RS = percent total residual solvents

% Water = percent water content

% ROI = percent residue on ignition

2.3 Storage and Requalification of Primary Reference Standard

The primary reference standard is stored at controlled room temperature (15°C to 30°C) and protected from light. Annual requalification is currently performed for attributes related to stability. Results for the first and second re-qualifications are provided in Table 1. Requalification intervals may be revised based on analysis of stability data.

2.4 Procedure for Qualifying New Batches of Primary Reference Standard

When the current batch of primary reference standard (Lot XXXXXXXXXX) is depleted, a new batch will be qualified by the tests indicated in Table 2. Where the results of tests meet the acceptance criteria in Table 2, the reference standard will be considered qualified.

Table 1. Results for Analysis of INN Primary Reference Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results	Requalification	Requalification
Testing date			July 2012	June 2013	June 2014
Appearance[a]	White to off-white powder	Visual evaluation	White powder	White powder	White powder
Identification (FT-IR)[a]	Consistent with structure at release; Conforms to standard at requalification	FT-IR	Consistent with structure	Conforms to standard	Conforms to standard
Identification (NMR)	Consistent with structure	NMR spectrometry	Consistent with structure	Not tested	Not tested
Identification (ESI-MS)	M = XXXX.X ± X.X u (monoisotopic mass)	Electrospray ionization-mass spectrometry	XXXX	Not tested	Not tested
Organic Impurities (w/w%)[a, b]		HPLC			
Report each substance ≥ 0.10%					
Impurity 1	≤ X.X		< X.XX	< X.XX	< X.XX
Impurity 2	≤ X.X		< X.XX	< X.XX	< X.XX
Impurity 3	≤ X.X		< X.XX	< X.XX	< X.XX
Impurity 4	≤ X.X		< X.XX	< X.XX	< X.XX
Impurity 5	≤ X.X		< X.XX	< X.XX	< X.XX
Impurity 6	≤ X.X		< X.XX	< X.XX	< X.XX
Impurity 7	≤ X.X		< X.XX	< X.XX	< X.XX
Total Impurities	≤ X.X		X.XX	X.XX	X.XX

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[a] Performed for requalification

[b] Acceptance criteria were in area% at the initial qualification

Table 1. Results for Analysis of INN Primary Reference Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results	Requalification	Requalification
Chiral Impurities (w/w%) [a]		Chiral HPLC			
Impurity A (R, M Isomer)	≤ XXX		< X.XX	< X.XX	< X.XX
Impurity B (S, P Isomer)	≤ XX		< X.XX	< X.XX	< X.XX
Impurity C (R, P Isomer)	≤ XX		< X.XX	< X.XX	< X.XX
Total Chiral Impurities	≤ XX		< X.XX	< X.XX	< X.XX
Water content[a] (w/w%)	Report	Karl Fischer (KF) titration	X.X	X.X	X.X
Residual solvents (ppm)		GC			
Solvent 1	Report		< XX		
Solvent 2	Report		< XX		
Solvent 3	Report		< XX		
Solvent 4	Report		< XX		
Solvent 5	Report		< XX		
Solvent 6	Report		XXXX	Not tested	Not tested
Solvent 7	Report		Not detected		
Solvent 8	Report		Not detected		
Solvent 9	Report		Not detected		
Solvent 10	Report		< XXX		
Solvent 11	Report		< XXX		
Total Solvents	Report		XXXX		

[a] Performed for requalification
[b] Acceptance criteria were in area% at the initial qualification

Table 1. Results for Analysis of INN Primary Reference Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results	Requalification	Requalification
Elemental Impurities	Report	ICP-MS	As, Cd, Hg, Pb: Not detected Pd: < X ppm	Not tested	Not tested
Residue on Ignition (w/w%)	≤ XX	USP < 281 >	< X.X	Not tested	Not tested
Differential Scanning Calorimetry	Report	Differential Scanning Calorimetry	Endotherm onset at XXX°C	Not tested	Not tested
X-Ray Powder Diffraction	Consistent with the Form X	X-Ray Powder Diffraction	Consistent	Not tested	Not tested
Quantitative NMR (w/w%)[c]	Report	NMR Spectrometry	XX.XX	Not tested	Not tested
Assigned Purity (w/w%)[a]	Report	Calculation (Section 2.2)	XX.XX	XX.XX	XX.XX

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[a] Performed for requalification
[b] Acceptance criteria were in area% at the initial qualification

Table 2. Specification for Future Batches of INN Primary Reference Standard

Test	Acceptance Criteria
Appearance[a]	White to off-white powder
Identification (FT-IR)[a]	Conforms to standard
Identification (MS)	Conforms to structure
Identification (NMR)	Conforms to structure
Organic Impurities (HPLC)[a]	Impurity 1 ≤ X.X w/w% Impurity 2 ≤ X.X w/w% Impurity 3 ≤ X.X w/w% Impurity 4 ≤ X.X w/w% Impurity 5 ≤ X.X w/w% Impurity 6 ≤ X.X w/w% Impurity 7 ≤ X.X w/w%[b] Impurity 8 ≤ X.X w/w%[b] Impurity 9 ≤ X.X w/w%[b] Any single unspecified impurity ≤ X.X %w/w Total Impurities ≤ X.X w/w%
Chiral Impurities (HPLC)	Impurity A (R,M Isomer) ≤ X.X w/w% Impurity B (S,P Isomer) ≤ X.X w/w% Impurity C (R,P Isomer) ≤ X.X w/w% Total Chiral Impurities ≤ X.X w/w%
Water Content (KF Titration)[a]	Report
Residual Solvents (GC)	Solvent 1: ≤ XXXX ppm Solvent 2: ≤ XXX ppm Solvent 3: ≤ XXX ppm
Elemental Impurities (ICP-MS)	As, Cd, Hg, Pb, Ni, Co, V, Pd: Report
Residue on Ignition	≤ X.X w/w%
X-Ray Powder Diffraction	Conforms to Form X
Quantitative NMR	XX.X w/w%
Assigned Purity[a]	Calculation (Section 2.2) XX.X w/w%

[a] Performed for requalification
[b] Impurities to be included in alignment with the CP-X.X drug substance specification for future qualification and requalification of INN Primary Reference Standard

3. Working Reference Standard

3.1 Working Reference Standard Preparation

The working (secondary) reference standard (Lot XXXXXXXXXX) for INN was qualified using primary reference standard (Lot XXXXXXXXXX). The results from testing performed for initial release of the working reference standard are provided in Table 3.

The calculation of INN content in the working reference standard was determind by applying the mass balance principle and assigned a purity using the same formula as for primary reference standard assigned purity determination (Section 2.2).

3.2 Procedure for Qualifying New Lots of Working Reference Standard

When the current batch of working reference standard ((Lot XXXXXXXXXX) is depleted, a new batch will be qualified by the tests indicated in Table 3. Where the results of tests meet the acceptance criteria in Table 3, the reference standard will be considered qualified.

3.3 Working Reference Standard Storage and Requalification

The working reference standards are stored at controlled room temperature (15°C to 30°C) and protected from light. Requalification will be performed annually on material remaining in stock. The results of hitherto existing requalification are provided in Table 3. Requalification intervals may be revised based on analysis of stability data.

Table 3. Results for Analysis of INN Working Reference Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results	Requalification
Testing date			August 2020	
Appearance [a]	White to off-white to yellow to light brown powder	Visual	Off-white powder	
Identification by FT-IR [a]	Conforms to standard	FT-IR	Conforms to standard	
Identification by UV/Vis	Conforms to standard Report Average Molar Extinction Coefficient for each peak	UV/Vis	Conforms to standard Average ε(XXX nm): XXXXX Average ε(XXX nm): XXXXX	
Identification by NMR	Consistent with structure	^{1}H NMR	Consistent with structure	
Identification by Mass Spectrometry	Consistent with structure	Mass Spectrometry	Consistent with structure	
Identification (Stereoisomer RT)	Conforms to standard	Chiral HPLC	Conforms to standard	
Elemental Impurity (ppm)		ICP-MS		
As	≤ X.X		< X.X	
Cd	≤ X.X		< X.X	
Pb	≤ X.X		< X.X	
Hg	≤ X		< X.X	
Pd	≤ XX		< X.X	
Ni	≤ XX		< X.X	
Co	≤ XX		< X	
V	≤ XX		< X	
TFA Content by Ion Chromatography (ppm)	≤ XXXX	Ion Chromatography	< LOQ (XXX)	

[a] Performed for requalification

Table 3. Results for Analysis of INN Working Reference Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results	Requalification
Particle Size Distribution (µm)		PSD		
D90	≤ XX		XX	
Water Content (w/w%)	≤ X.X	KF	X.X	
Chiral Impurities (w/w%)		Chiral HPLC		
Impurity A (R,M Isomer)	≤ X.X		< X.XX	
Impurity B (S,P Isomer)	≤ X.X		< X.XX	
Impurity C (R,P Isomer)	≤ X.X		< X.XX	
Total Chiral Impurities	≤ X.X		< X.XX	
Organic Impurities (w/w%)[a]		HPLC		
Impurity 1	≤ X.X		< X.XX	
Impurity 2	≤ X.X		X.XX	
Impurity 3	≤ X.X		< X.XX	
Impurity 4	≤ X.X		< X.XX	
Impurity 5	≤ X.X		< X.XX	
Impurity 6	≤ X.X		< X.XX	
Impurity 7	≤ X.X		< X.XX	
Single unspecified impurity	≤ X.X		< X.XX	
Total Impurities	≤ X.X		< X.XX	

[a] Performed for requalification

Table 3. Results for Analysis of INN Working Reference Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results	Requalification
Residual Solvents (ppm)		GC		
Solvent 1	≤ XXX		< LOQ (XX)	
Solvent 2	≤ XXXX		< LOQ (XXX)	
Solvent 3	≤ XXXX		< LOQ (XXX)	
Solvent 4	≤ XXXX		< LOQ (XXX)	
Solvent 5	≤ XXXX		XXX	
Solvent 6	≤ XXX		< LOQ (XXX)	
Residue on Ignition (w/w%)	≤ X.X	ROI	X.X	
Assigned Purity (%)[a]	Calculated	-	XX.XX	

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[a] Performed for requalification

4. Achiral Resolution Standards

4.1 Achiral Resolution Standard Preparation

<Number of> impurity standards are synthesized and used to prepare the INN achiral resolution standard used in HPLC testing. For structural information, refer to 3.2.S.3.2 (Impurities).

4.2 Procedure for Qualifying Achiral Resolution Standard

When the batch of achiral resolution standard containing impurities described in Table 4 is depleted, a new batch will be qualified by the tests indicated in Table 4.

Table 4. Results for INN Achiral Resolution Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results
Testing date			May 2020
Appearance [a]	Report	Visual	Off-white powder
Identification (LC Retention Time)	Conforms to impurity standard by LC Retention Time	HPLC	Conforms
Purity (area%) [a]	Report	HPLC	XX.X
Organic Impurities (area%)[a]		HPLC	
Impurity 1	Report		X.XX
Impurity 2	Report		X.XX
Impurity 3	Report		X.XX
Impurity 4	Report		X.XX
Impurity 5	Report		X.XX
Impurity 6	Report		X.XX
Impurity 7	Report		X.XX
Total Impurities	Report		X.XX

[a] Performed for requalification

5. Chiral Resolution Standards

5.1 Chiral Resolution Standard Preparation

<Number of> chiral impurity standards are synthesized and used to prepare the INN chiral resolution standard used in chiral impurity testing by chiral HPLC. For structural information, refer to 3.2.S.3.2 (Impurities).

5.2 Procedure for Qualifying Chiral Resolution Standard

When the batch of chiral resolution standard containing impurities described in Table 5 is depleted, a new batch will be qualified by the tests indicated in Table 5.

Table 5. Results for INN Chiral Resolution Standard (Lot XXXXXXXXXX)

Test	Acceptance Criteria	Method	Initial Results
Testing date			October 2019
Appearance[a]	Report	Visual	White powder
Identification (LC Retention Time)	Conforms to impurity standard by LC Retention Time	HPLC	Conforms
Purity (area%)[a]	Report	HPLC	X.XX
Chiral Impurities (area%)[a]		HPLC	
Impurity A (R,M Isomer)	Report		X.XX
Impurity B (S,P Isomer)	Report		X.XX
Impurity C (R,P Isomer)	Report		X.XX

[a] Performed for requalification