3.2.S.2.4 Control of Critical Steps and Intermediates

The manufacture process are well controlled by an overall strategy of CPPs (Critical Process Parameters) monitoring, IPCs (In-process Controls) Testing and Intermediates release testing. The details are described in following sections.

3.2.S.2.4.1 Critical Process Parameters

The critical steps, process parameters and their control ranges were first identified during development and were further confirmed through scale up manufacturing. These critical parameters are properly controlled during the submission batches manufacture and will be controlled during the commercial manufacture.

The critical process parameters (CPPs) are considered to have significant impact on quality, yield, and process performance. The acceptance criteria of CPPs as well as justification are summarized in Table 3.2.S.2.4-1.

Table 3.2.S.2.4-1Critical Parameters and Acceptance Criteria

Processes	Critical Process Parameters	Acceptance Criteria	Justification
Step I: Alkylation reaction	Reaction temperature	85 ± 2°C	The reaction cannot complete under lower temperature and impurities will increase at higher temperature and further impact the impurity profile of the final product.
Step III: Deprotonation reaction	Reaction temperature	5 ± 2°C	The reaction cannot complete under lower temperature, while impurity A will increase under high temperature, which will influence the quality of intermediate, in sequence, affect the quality of final product.
	pH value	2.7 - 3.2	pH too high will lead incomplete precipitation of IRB03, while too low will lead impurity to precipitate along with IRB03, and further impact the impurity profile of the final product
Step IV: Purification	Crystallization temperature	0 ± 5°C	To produce the desired crystal form and increase the purified yield.
Step V: Drying and Grinding	Drying temperature	60 ± 5°C	It will impact the crystal form and residual solvents in the final product.
Step VI: Blending and Packing	Blending time	20 ± 1 min	It will impact the content uniformity of the final product.

3.2.S.2.4.2 In Process Control

1. In Process Tests of Each Step

The tests used to monitor the process progress and reaction end-points of each step are listed in Table 3.2.S.2.4-2.

3.2.S.2.4-2 In-Process Control Information

Process	In-process tests	Acceptance Criteria	Analytical Method
Step I: Alkylation reaction	Reaction end-point	Un-reacted BBTT < 0.5%	HPLC
Step II: Deprotection reaction	Reaction end-point	Unreacted IRB 01 ≤ 0.02%	TLC
Step III: Deprotonation reaction	Drying end-point	Water content of IRB03a <10.0%	Karl Fischer
	Drying end-point	Water content IRB03 <6.0%	Karl Fischer
Step V: Drying and Grinding	Drying end-point of IRB05	Water content of IRB05< 0.5%	Karl Fischer
		Residual ethanol in IRB05 <5000 ppm	GC

2. Analytical Procedures for In-process Testing

2.1 Analytical Procedure for Step I

Take 1 drop of the original reactant and dilute with acetonitrile to about 4 mL as the test solution. Proceed by using the HPLC method described bellowed. The content of BBTT should be less than 0.5%.

Chromatographic System

Column	Agilent ZORBAX Rx-C8 (150 mm × 4.6 mm, 5 μm)
Column Temperature	25°C
Detector	UV at 220 nm
Flow rate	1.0 mL/min

Mobile Phase: pH=3.2 phosphoric acid buffer solution (5.5 mL of phosphoric acid is diluted with 950 mL of high purity water, and then adjusted with triethylamine until pH is 3.2): acetonitrile=30:70

2.2 Analytical Procedure for the Step II

Take original reactant as the test solution and the original IRB01 solution from Step I as the standard solution. Apply 1 drop of the test solution and 1 drop of the standard solution by capillary to a suitable thin-layer chromatographic silica gel, about 1 cm from the bottom edge of the plate. Develop the chromatogram in a separation chamber containing a solvent system of ethyl acetate and petroleum ether (2:3) to a depth of about 1 cm. When the solvent front has crossed about 80% of the plate, remove the plate and dry it. Examine the plate under UV light at 254 nm. The trace of the IRB01 on the silica gel produced by the test solution should disappear.

2.3 Analytical Procedure for Step III

Water content of IRB03a: Take 0.2-0.5 g of IRB03a and proceed by method for analyzing the water content of IRB05 described in Section *3.2.S.2.4.3 Intermediates*. The water content of IRB03a should be not more than 10.0%.

Water content of IRB03: Take 0.2-0.5 g of IRB03 and proceed by method for analyzing the water content of IRB05 described in Section *3.2.S.2.4.3 Intermediates*. The water content of IRB03a should be not more than 6.0%.

2.4 Analytical Procedure for Step IV

Water content of IRB05: Take about 1.0 g of IRB05 and proceed by method for analyzing the water content of IRB05 described in Section *3.2.S.2.4.3 Intermediates*. The water content of IRB05 should be not more than 0.5%.

Residual ethanol in IRB05: take suitable amount of IRB05 and proceed by method for analyzing the residual ethanol in IRB05 described in Section *3.2.S.4.2 Analytical Procedures*. The residual ethanol in IRB05 should be not more than 5000ppm.

3.2.S.2.4.3 Intermediates

Four intermediates (IRB02, IRB03, IRB04, IRB05) are isolated and three intermediates (IRB03, IRB04, IRB05) are proposed to be controlled during the manufacturing of commercial batches.

IRB02 was monitored during development stage and submission batches production. It is for investigation purpose and will be not for routine controlling during commercial manufacture.
The acceptance criteria and test methods for all isolated intermediates are provided below.

(a) Analytical Procedure for IRB02 (For investigation purpose while not for routine controlling during commercial manufacture)

Specification of IRB02 is listed in the following table.

Table 3.2.S.2.4-3 Specification for Intermediate IRB02

Test		Acceptance Criteria	Analytical Procedure
Related substances	Impurity 2-3(RRT ≈ 0.95)	Not more than 0.10%	HPLC
	Impurity 2-11(RRT ≈ 1.52)	Not more than 0.10%	
	Total impurities	Not more than 10.0%	
Purity		Not less than 90.0%	

Analytical Methods

Related substances and purity

Reagents

triethylamine
Phosphoric acid
Acetonitrile
Purity water

Chromatographic Conditions

Column	Agilent Eclipse Plus C18 (250 mm × 4.6 mm, 5 μm)
Column Temperature	25°C
Detector	UV at 220 nm
Flow rate	1.0 mL/min
Injection volume	5 μL
Run time	28 min

Mobile Phase:

Time (min)	pH=3.2 Phosphoric Acid Buffer Solution (%)	Acetonitrile (%)
0	60	40
18	20	80
28	20	80

pH=3.2 phosphoric acid buffer solution: 5.5 mL of phosphoric acid is diluted with 950 mL of purity water, and then adjusted with triethylamine until pH is 3.2.

Solution Preparation

Blank Solution: acetonitrile/water (1:1)
Test Solution: Weigh about 50 mg of sample and transfer to a 50 mL volumetric flask. The sample is dissolved and diluted with Blank Solution to volume. Mix well.

Procedure

Separately single inject 5 µL of the Blank Solution and Test Solution into the chromatograph and record the chromatogram. The resolution, R, between principle peak and its adjacent peak should be not 1.5.

Measure the area of each impurity peak and calculate the percentages of individual impurities and purity using the Area Normalization Method, excluding the solvent peak.

Acceptance criteria: each of impurity 2-3 (RRT ≈ 0.95) and impurity 2-11 (RRT ≈ 0.1.52) should be not more than 0.10%, total impurities should be not more than 10.0%; purity should be not less than 90.0%.

(b) Analytical Procedure for IRB03

Specification of IRB03 is listed in the following table.

Table 3.2.S.2.4-4 Specification for Intermediate IRB03

Test		**Acceptance Criteria**	**Analytical Procedure**
Appearance		Almost white solid	Visual examination
Water Content		Not more than 6.0%	Karl Fisher
Related substances	Impurity 1(RRT ≈ 0.95)	Not more than 0.10%	HPLC
	Impurity 2 (RRT ≈ 1.52)	Not more than 0.10%	
	Impurity 3 (RRT ≈ 0.79)	Not more than 0.10%	
Purity		Not less than 90.0%	

Analytical Methods

Appearance

Visually examine the material and record physical state. It should be almost white solid.

Water Content

Reagents

Anhydrous methanol

Karl Fischer reagent

Procedure

Take about 0.3-0.5 g of the sample and dissolve in anhydrous methanol. Proceed as directed in the *Ph.Eur.2.5.12.*

Test two samples in parallel. Calculate the arithmetic mean value of the two results as the test result. The water content is IRB03 should be not more than 6.0%.

Related substances and purity

Reagents

triethylamine
Phosphoric acid
Acetonitrile
Purity water

Chromatographic Conditions

Column	Agilent Eclipse Plus C18 (250 mm × 4.6 mm, 5 μm)
Column Temperature	25°C
Detector	UV at 220 nm
Flow rate	1.0 mL/min
Injection volume	5 μL
Run time	28 min

Mobile Phase:

Time (min)	pH=3.2 Phosphoric Acid Buffer Solution (%)	Acetonitrile (%)
0	60	40
18	20	80
28	20	80

pH=3.2 phosphoric acid buffer solution: 5.5 mL of phosphoric acid is diluted with 950 mL of purity water, and then adjusted with triethylamine until pH is 3.2.

Solution Preparation

Blank Solution: acetonitrile/water (1:1)
Test Solution: Weigh about 50 mg of sample and transfer to a 50 mL volumetric flask. The sample is dissolved and diluted with Blank Solution to volume. Mix well.

Procedure

Separately single inject 5 μL of the Blank Solution and Test Solution into the chromatograph and record the chromatogram. The resolution, R, between principle peak and its adjacent peak should be not 1.5.

Measure the area of each impurity peak and calculate the percentages of individual impurities and purity using the Area Normalization Method, excluding the solvent peak.

Acceptance criteria: each of impurity 1 (RRT ≈ 0.95), impurity 2 (RRT ≈ 0.1.52) and impurity 3 should be not more than 0.10%; purity should be not less than 90.0%.

(c)Analytical Procedure for IRB04

Specification of IRB03 is listed in the following table.

Table 3.2.S.2.4-5 Specification for Intermediate IRB04

Test	Acceptance Criteria	Analytical Procedure
Appearance	Almost white solid	Visual examination
Foreign Matter	Not more than 10/g	

Analytical Methods

Appearance

Visually examine the material and record the color. It should be almost white powder.

Foreign Matter

Weigh about 1.0 g of the sample and dissolve with 10 mL of dimethylformamide. Observe the solution and determine the foreign matter particle count, which should not exceed 10/g.

(d)Analytical Procedure for IRB05

Specification of IRB03 is listed in the following table.

Table 3.2.S.2.4-6 Specification for Intermediate IRB05

Test		**Acceptance Criteria**	**Analytical Procedure**
Appearance		White or almost white solid	Visual examination
Identification		Infrared spectrum is concordant with that of the reference standard	IR
Water Content		Not more than 0.5%	Karl Fisher
Related Substances	Impurity A	Not more than 0.15%	HPLC
	Any other impurity	Not more than 0.10%	
	Total impurities	Not more than 0.2%	

Analytical Methods

A summary of the analytical procedures is provided below.

Appearance

Visually examine the material and record the colour. It should be white or almost white solid.

Water Content

Reagents

Anhydrous methanol

Karl Fischer reagent

Procedure

Take about 1.0 g of the sample and dissolve with anhydrous methanol. Proceed as directed in *Ph.Eur.2.5.12*.

Test two samples in parallel. Calculate the arithmetical mean value of the two results as the test result. It should be not more than 0.5%.

Identification

Throughly mix about 1-2 mg of the sample with 300-400 mg of potassium bromide. Record the spectra of the test specimen and the irbesartan reference standard over the range from 4000 cm^{-1} to 400 cm^{-1}. The IR absorption spectrum of the preparation of the test specimen should be concordant with that of reference standard.

Related substances

Reagents

Triethylamine
Phosphoric acid
Acetonitrile
Purity water
pH=3.4 Phosphoric Acid Buffer Solution: 5.5 mL of phosphoric acid is diluted with 950 mL of water, and adjusted with triethylamine until pH is 3.4, filter and degass.

Chromatographic System

Column	MNEC 250/4 NUCLEOSIL 100-5 C_{18} (1.0 mm × 250 mm, 5 μm, 100A)
Column Temperature	25 °C
Detector	UV at 220 nm
Flow rate	1.0 mL/min
Injection volume	10 μL
Run time	38 min
Mobile Phase	pH=3.4 Phosphoric acid buffer solution : acetonitrile = 67 :33

Solution Preparation

Blank Solution: methanol

Standard Solution: Accurately weigh 21 mg each of Irbesartan RS and USP Irbesartan Related Compound A RS, and transfer to a 50 mL volumetric flask. Dissolve and dilute with methanol to volume, mix well. Pipette 3 mL of this solution to a 25 mL of volumetric flask, dilute with methanol to volume, mix well. Prepare Standard Solution 1 and Standard Solution 2 in parallel.

Test Solution: Accurately weigh about 50 mg of sample, transfer to a 50 mL of volumetric flask, dissolve and dilute with methanol to volume, mix well. Prepare Test Solution 1 and Test Solution 2 in parallel.

Sensitive Solution: Pipette1 mL of Standard Solution to a 100 mL volumetric flask, dilute with methanol to volume. Mix well.

Procedure

Follow the injection sequence as defined in Table 3.2.S.2.4-6. Record the chromatograms.

Table 3.2.S.2.4-7 Injection sequence-related substances

Sequence	Frequency of Injection
Blank Solution	1-2
Standard Solution 1	5
Standard Solution 2	2
Sensitive Solution	1
Test Solution 1	1
Test Solution 2	1
…	1
Standard Solution 1 (every 7-8 h)	1
…	1
Standard Solution 1	1
Sensitive Solution	1

System Suitability Requirements

In the chromatogram obtained with Standard Solution 1, the Resolution, R, between the peaks of irbesartan and impurity A should be not less than 3.0, and the tailing factor of peak due to irbesartan should be 0.8~1.5. The relative standard deviation (RSD) of the irbesartan peak area and the impurity A peak area from five relicate injections should be not more than 2.0%.the signal-to-noise ratio (S/N) of the irbesartan peak and impurity A peak in the Sensitive Solution should be not less than 10.

Calculation

Calculate the percentage of impurity A in the portion of irbesartan according to the formula below.

$$\text{Assay}(\%) = \frac{W_{s1} \times D_u \times r_u \times P}{D_{s1} \times W_u \times r_{s1}} \times 100\%$$

In which,

W_{s1}: Weight of USP Irbesartan Related Compound A RS used in preparation of Standard Solution 1, mg;

W_u: Weight of irbesartan used in preparation of Test Solution, mg;

r_{s1}: Average area of impurity A peaks obtained from two single injection of Standard Solution 1 before and after the Test Solution;
r_u: Peak area of impurity A obtained from Test Solution;
D_u: Diluting factor of Test Solution, mL;
D_{s1}: Diluting factor of Standard Solution 1, mL;
P: Purity of Irbesartan Related Compound A RS, %;

Calculate the percentage of any other impurity in the portion of irbesartan according to the formula below.

$$\text{Assay}(\%) = \frac{W_{s1} \times D_u \times r_u \times P}{D_{s1} \times W_u \times r_{s1}} \times 100\%$$

In which,

W_{s1}: Weight of Irbesartan WRS used in preparation of Standard Solution 1, mg;
W_u: Weight of irbesartan used in preparation of Test Solution, mg;
r_{s1}: Average area of irbesartan peaks obtained from two single injection of Standard Solution 1 before and after the Test Solution;
r_u: Peak area of any other impurity obtained from Test Solution;
D_u: Diluting factor of Test Solution, mL;
D_{s1}: Diluting factor of Standard Solution 1, mL;
P: Purity of Irbesartan WRS, %;

Acceptance Critera: Impurity A should be not more than 0.15%; any other impurity should be not more than 0.10%, total impurities should be not more than 0.2%.