

Specification for Mirogabalin Besilate-EDMF

Test Items	Limits	Test Method
Appearance	White to pale yellowish-white powder	Visual
Identification	Infrared absorption spectrum corresponds to the spectrum obtained with the Mirogabalin besilate RS.	Ph.Eur. 2.2.24
	The retention time of the major peak in the chromatogram of the <i>Sample solution</i> corresponds to that in the chromatogram of the <i>Standard solution</i> , as obtained in the <i>Assay</i> .	In-house HPLC method
Water	≤0.5%	Ph.Eur. 2.5.12
Sulfate ash	≤0.1%	Ph.Eur. 2.4.14
Related substances 1 (HPLC)	Mirogabalin acid≤0.15%	In-house HPLC method
	Mirogabalin epimer≤0.15%	
	Any unspecified impurity≤0.10%	
	Total impurities≤0.5%	
Related substance 2 (LC-MS)	Mirogabalin reduced impurity≤0.15%	In-house LC-MS method
Isomer (HPLC)	Mirogabalin enantiomer≤0.15%	In-house HPLC method
Assay (HPLC)	98.0%-102.0% (Calculated on anhydrous and solvent-free basis)	In-house HPLC method
Besilate content	40.9%-45.2%	In-house titration method
Residual solvents (GC)	Ethanol≤5000ppm	In-house GC method
	Toluene≤890ppm	
	Tetrahydrofuran≤720ppm	
	<i>tert</i> -Butylmethyl ether≤5000ppm	

Mirogabalin acid: 2-((1R,5S,6S)-3-ethyl-6-(nitromethyl)bicyclo[3.2.0]hept-3-en-6-yl)acetic acid

Mirogabalin epimer: 2-((1R,5S,6R)-6-(aminomethyl)-3-ethylbicyclo[3.2.0]hept-3-en-6-yl)acetic acid

Mirogabalin reduced impurity: 2-((1R,5R,6S)-6-(aminomethyl)-3-ethylbicyclo[3.2.0]heptan-6-yl)acetic acid

Mirogabalin enantiomer: 2-((1S,5R,6R)-6-(aminomethyl)-3-ethylbicyclo[3.2.0]hept-3-en-6-yl)acetic acid

Analytical Procedures of Mirogabalin Besilate

1.0 APPEARANCE

By visual examination.

2.0 IDENTIFICATION

➤ IR

Ph.Eur.2.2.24: Examine by infrared absorption spectrophotometers, comparing with the spectrum obtained with Reference Standard.

➤ HPLC retention time

The retention time of the major peak in the chromatogram of the Sample solution corresponds to that in the chromatogram of the *Standard solution*, as obtained in the Assay.

3.0 WATER

Ph. Eur. 2.5.12: Not more than 0.5%, determined on 0.5 g of the substance to be examined by Karl-Fischer method.

4.0 SULFATE ASH

Ph.Eur.2.4.14: Not more than 0.1%, determined on 1.0 g.

5.0 BESILATE CONTENT

Dissolve 0.2g of substance to be examined in 60 mL of water. Titrate with 0.1 mol/L of sodium hydroxide titration solution, determining the end-point potentiometrically. Carry out a blank titration. 1mL of 1.0 mol/L sodium hydroxide is equivalent to 158.18mg of C₆H₆O₃S.

Calculate the content of C₆H₆O₃S taken:

$$\text{Result} = \frac{(V - V_0) \times C \times 158.18}{m \times 1000} \times 100\%$$

In which, V is the volume of sodium hydroxide titrant consumed at the end of the titration (mL);

V₀ is the volume of sodium hydroxide titrant consumed by blank titration;

C is the concentration of the sodium hydroxide titrant (mol/L);

158.18 is mean 1mL of 1.0 mol/L sodium hydroxide is equivalent to 158.18mg of C₆H₆O₃S;

m is the weight of the substance to be examined.

1000 is the unit conversion factor.

6.0 RELATED SUBSTANCES 1 (HPLC)

➤ Chromatographic system

Instrument	HPLC-system equipped with UV detector		
Column	Waters XSelesct CSH Fluoro-Phenyl, 150×4.6 mm, 3.5μm		
Mobile phase A	Dissolve 1.36g of monobasic potassium phosphate in 1000 mL of water.		
Mobile phase B	Methanol		
Detection wavelength	210nm		
Flow rate	1.0mL/min		
Injection vol.	5μl		
Column temp.	40°C		
Run time	40min		
Gradient	Time (min.)	Mobile phase A (%V/V)	Mobile phase B (%V/V)
	0	95	5
	10	74	26
	27	30	70
	32	30	70
	32.1	95	5
	40	95	5

➤ Solution Preparation

Diluent: methanol: water (50:50, V/V)

Blank: Diluent.

System suitability solution: 12μg/mL of Mirogabalin acid RS, 12μg/mL of Mirogabalin epimer RS and 8.0mg/mL of Mirogabalin Besilate RS in diluent.

Reference solution: 8.0μg/mL of Mirogabalin Besilate RS in diluent.

Sensitivity solution: 4.0μg/mL of Mirogabalin Besilate RS in diluent.

Sample solution: 8.0mg/mL of substance to be examined in diluent.

➤ Procedure

Inject one injection of *blank solution*, one injection of *sensitivity solution*, replicate six injections of *reference solution* and one injection of *system suitability solution*; then perform one injection of *sample solution* after the system suitability is set-up; record the chromatograms and results.

➤ **Suitability requirements**

S/N: NLT 10 of major peak obtained from *sensitivity solution*.

RSD: NMT 5.0% for major peak area obtained from replicate injections of *reference solution*.

Resolution: NLT 1.5 between the major peak, known impurities and adjacent peaks in *system suitability solution*.

➤ **Calculation**

Calculated the percentage content of impurity according to the following formula:

$$\text{Result} = \frac{A_t \times C_r}{A_r \times C_t} \times F \times 100\%$$

In which, A_t is the peak area of each impurity obtained from the sample solution;

C_r is the concentration of the reference solution (mg/mL).

A_r is the A_v peak area of major peak obtained from the reference solution;

C_t is the concentration of the sample solution (mg/mL);

F is the correction factor.

The RRT and correction factor of each impurity are as follows:

Impurity name	RRT	Correction factor
Mirogabalin	1.0	——
Mirogabalin epimer	1.08	0.46
Mirogabalin acid	2.63	0.38
Any unspecified impurity	——	1.0

7.0 RELATED SUBSTANCES 2 (MIROGABALIN REDUCED IMPURITY) (LC-MS)

➤ Chromatographic system

Instrument	HPLC-system equipped with a single quadrupole mass spectrometer detector		
Column	ACE UltraCora 2.5 Super C18, 30×2.1mm		
Mobile phase A	0.1% Trifluoroacetic acid solution		
Mobile phase B	0.1% Trifluoroacetic acid acetonitrile solution		
Flow rate	1.0mL/min		
Injection vol.	1μl		
Column temp.	50°C		
Run time	7min		
Gradient	Time (min.)	Mobile phase A (%V/V)	Mobile phase B (%V/V)
	0	98	2
	4	80	20
	5	80	20
	5.1	98	2
	7	98	2
ESI Source Parameters		MS Detection Parameters	
Dry Gas Temperature	350°C	Scan Mode	SIM
Nebulizer Gas Pressure	35psi	SIM Ion Current	212 m/z (M+H ⁺)
Capillary Voltage	3000V	Fragmentor Voltage	70V
Ionization Mode	Positive		

➤ Solution Preparation

Diluent: methanol: water (50:50, V/V)

Blank: Diluent.

Standard solution: 0.15μg/mL of Mirogabalin reduced impurity RS in diluent.

Sample solution: 0.1mg/mL of substance to be examined in diluent.

➤ Procedure

Inject one injection of *blank solution*, replicate six injections of *standard solution*; then perform one injection of *sample solution* after the system suitability is set-up; record the chromatograms and results.

➤ **Suitability requirements**

RSD: NMT 15.0% for major peak area obtained from replicate injections of *standard solution*.

➤ **Calculation**

Mirogabalin reduced impurity is calculated according to the following formula:

$$\text{Result} = \frac{A_t \times C_s \times P}{A_s \times C_t} \times 100\%$$

In which, A_t is the peak area of Mirogabalin reduced impurity obtained from the sample solution;

C_s is the concentration of the standard solution (mg/mL).

A_s is the Av. peak area of Mirogabalin reduced impurity obtained from the standard solution;

C_t is the concentration of the sample solution (mg/mL);

P is the assay of impurity RS.

8.0 ISOMER (MIROGABALIN ENANTIOMER) (HPLC)

➤ Chromatographic system

Instrument	HPLC-system equipped with UV detector
Column	CHIRALPAK AGP 150mm×4.0mm, 5μm
Buffer	Dissolve 1.36g of monobasic potassium phosphate and 1.74g of dibasic potassium phosphate in 1000 mL of water, adjust the pH to 6.0 with perchloric acid
Mobile phase	Buffer : methanol = 97:3 (V/V)
Detection wavelength	215nm
Flow rate	0.3mL/min
Injection vol.	5μL
Column temp.	30°C
Run time	30min

➤ Solution Preparation

Diluent: water: acetonitrile (98:2, V/V).

Blank: Diluent.

System suitability solution: 3.75 μg/mL of Mirogabalin enantiomer RS and 2.5 mg/mL of Mirogabalin besilate in diluent.

Reference solution: 3.75 μg/mL of Mirogabalin besilate in diluent.

Sample solution: 2.5 mg/mL of substance to be examined in diluent.

➤ Procedure

Inject one injection of *blank solution*, replicate six injections of *reference solution*, one injection of *system suitability solution*; then perform one injection of *sample solution* after the system suitability is set-up; record the chromatograms and results.

➤ Suitability requirements

RSD: NMT 5.0% for the major peak obtained from replicate injections of *reference solution*.

Resolution: NLT 1.5 between major peak and Mirogabalin enantiomer obtained from the *resolution solution*.

➤ **Calculation**

Mirogabalin enantiomer is calculated according to the following formula:

$$\text{Result} = \frac{A_t \times C_r}{A_r \times C_t} \times 100\%$$

In which, A_t is the peak area of Mirogabalin enantiomer obtained from the sample solution;

A_r is the Av. peak area of major peak obtained from the reference solution;

C_r is the concentration of reference solution (mg/mL).

C_t is the concentration of the sample solution (mg/mL);

9.0 ASSAY

➤ Chromatographic system

Instrument	HPLC-system equipped with UV detector		
Column	Waters XSelesct CSH Fluoro-Phenyl, 150×4.6 mm, 3.5μm		
Mobile phase A	Dissolve 1.36g of monobasic potassium phosphate in 1000 mL of water.		
Mobile phase B	Methanol		
Detection wavelength	210nm		
Flow rate	1.0mL/min		
Injection vol.	5μl		
Column temp.	40°C		
Run time	40min		
Gradient	Time (min.)	Mobile phase A (%V/V)	Mobile phase B (%V/V)
	0	95	5
	10	74	26
	27	30	70
	32	30	70
	32.1	95	5
	40	95	5

➤ Solution Preparation

Diluent: methanol: water (50:50, V/V)

Blank: Diluent.

Standard solution: 1.6mg/mL of Mirogabalin besilate RS in diluent.

Sample solution: 1.6mg/mL of substance to be examined in diluent.

➤ Procedure

Perform one injection of *blank solution*, replicate five injections of *Standard solution*, then perform one injection of sample solution after the system suitability is set-up; record the chromatograms and results.

➤ Suitability requirements

RSD: NMT 2.0% for the major peak obtained from replicate injections of *Standard solution*.

➤ **Calculation**

$$\text{Assay} = \frac{A_i \times C_r}{A_r \times C_i} \times \frac{P}{1 - W - R} \times 100\%$$

In which, A_i is the peak area of major peak obtained from the sample solution;

C_i is the concentration of the sample solution(mg/mL);

C_r is the concentration of the standard solution(mg/mL);

A_r is the Av. peak area of major peak obtained from the standard solution;

P is the assay of RS;

R is the residual solvent of the substance to be examined;

W is the water content of the substance to be examined.

10.0 RESIDUAL SOLVENTS

➤ Chromatographic System

Apparatus	GC equipped with FID detector and automatic headspace sampler		
Column	DB-624 30 m×0.32 mm, 1.8 μm		
Column Temp.	35°C for 7 min→120°C at 10°C/min and hold for 1 min→220°C at 40°C/min and hold for 6 min		
Flow rate	1.7 mL/min for 17min→2.5mL/min at 1.6mL/min and hold for 2 min		
Detector temperature	260°C	Injector temperature	140°C
Carrier Gas	N ₂	Split Ratio:	10:1
Vial temperature	75°C	Loop temperature	85°C
Trline temperature	100°C	Inject time	1.0min
Vial EQ time	30min	Injection volume	1.0 mL

➤ Solution Preparation

Diluent: N-Methylpyrrolidone (NMP)

Blank: Diluent, transfer 1.0 mL of diluent in 20 mL of head-space vial, cap and seal.

Standard solution: 500μg/mL of ethanol, 89μg/mL of toluene, 72μg/mL of tetrahydrofuran and 500μg/mL of tert-butylmethyl ether in Diluent, transfer 1.0 mL of the solution in 20 mL of head-space vial, cap and seal.

Sample solution: Accurately weigh 0.1g of substance to be examined into a 20 mL of head-space vial, then transfer 1.0 mL of diluent, cap, seal and mix.

➤ Procedure

Inject one injection of blank solution, replicate six injections of standard solutions, then perform one injection of sample solution after the system suitability is set-up; record the chromatograms and results.

➤ System suitability requirement

RSD: NMT 10.0% for each solvent obtained from *standard solution*.

Resolution: NLT 1.5 between each solvent in *standard solution*.

➤ Calculation

$$\text{Results} = \frac{R_u \times C_s}{C_u \times R_s}$$

In which, C_s is the concentration of each solvent in the standard solution(μg/mL);

C_u is the concentration of the sample solution(g/mL);

R_u is the peak area of each solvent obtained from the sample solution;

R_s is the Av. peak area of each solvent obtained from the standard solution;