## 1 Pregabalin

2 プレガバリン

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4 C<sub>8</sub>H<sub>17</sub>NO<sub>2</sub>: 159.23

5 (3S)-3-(Aminomethyl)-5-methylhexanoic acid

6 [148553-50-8]

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8 Pregabalin contains not less than 98.0% and not 9 more than 102.0% of pregabalin ( $C_8H_{17}NO_2$ ), calcu-10 lated on the anhydrous basis.

11 Description Pregabalin occurs as a white crystalline pow-12 der.

13 It is sparingly soluble in water, and slightly soluble in eth-14 anol (99.5).

15 Optical rotation  $[\alpha]_D^{20}$ :  $+10 - +12^\circ$  (0.2 g calculated 16 on the anhydrous basis, water, 20 mL, 100 mm).

17 Identification Determine the infrared absorption spectrum
18 of Pregabalin as directed in the potassium bromide disk
19 method under Infrared Spectrophotometry <2.25>, and com20 pare the spectrum with the Reference Spectrum or the spec21 trum of Pregabalin RS: both spectra exhibit similar intensities

22 of absorption at the same wave numbers.

23 Purity (1) Related substances—(i) Polar related substances-Dissolve 0.1 g of Pregabalin in the mobile phase to 24 25 make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase 26 27 to make exactly 10 mL. Pipet 1 mL of this solution, add the 28 mobile phase to make exactly 100 mL, and use this solution 29 as the standard solution. Perform the test with exactly 20  $\mu$ L 30 each of the sample solution and standard solution as directed 31 under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area,  $A_{\rm T}$ , of related 32 33 substances other than pregabalin in the sample solution and 34 the peak area, A<sub>s</sub>, of pregabalin in the standard solution by 35 the automatic integration method, and calculate the amount of each polar related substance by the following formula: 36 37 each polar related substance is not more than 0.10%. For the 38 peak area of related substance C, having the relative retention time of about 0.6 to pregabalin, multiply the correction factor 39 40 0.01.

41 Amount (%) of polar related substance  
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$$=A_T / A_S \times 1 / 10$$

43 Operating conditions—

44 Detector, column, column temperature, mobile phase, and

45 flow rate: Proceed as directed in the operating conditions in46 the Assay.

47 Time span of measurement: Until pregabalin is eluted, be-48 ginning after the solvent peak.

49 System suitability—

50 System performance and System repeatability: Proceed as51 directed in the system suitability in the Assay.

52 Test for required detectability: Pipet 5 mL of the standard 53 solution, and add the mobile phase to make exactly 10 mL. 54 Confirm that the peak area of pregabalin obtained with 20  $\mu$ L 55 of this solution is equivalent to 35 to 65% of that with 20  $\mu$ L 56 of the standard solution.

57 Nonpolar related substances-Dissolve 0.1 g of (ii) 58 Pregabalin in the mobile phase to make 10 mL, and use this 59 solution as the sample solution. Pipet 1 mL of the sample so-60 lution, add the mobile phase to make exactly 100 mL, and use 61 this solution as the standard solution. Perform the test with 62 exactly 20 µL each of the sample solution and standard solu-63 tion as directed under Liquid Chromatography <2.01> accord-64 ing to the following conditions. Determine each peak area, 65  $A_{\rm T}$ , in the sample solution and the peak area,  $A_{\rm S}$ , of pregabalin 66 in the standard solution by the automatic integration method, 67 and calculate the amount of each nonpolar related substance 68 by the following formula: the amount of the related substance 69 A having the relative retention time of about 2.4 to pregabalin 70 is not more than 0.15%, and the amount of each nonpolar re-71 lated substance other than related substance A is not more 72 than 0.10%. For the areas of the peaks of related substances 73 A and D, having the relative retention times of about 2.4 and 74 about 2.8 to pregabalin, multiply their correction factors, 0.05 75 and 0.01, respectively.

Amount (%) of nonpolar related substance = $A_{\rm T}/A_{\rm S}$ 

78 Operating conditions—

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Detector, column, column temperature, and flow rate: Pro-ceed as directed in the operating conditions in the Assay.

81 Mobile phase: Dissolve 3.40 g of potassium dihydrogen 82 phosphate in 1000 mL of water, and adjust to pH 6.3 with 83 ammonia solution (28). To 450 mL of this solution add 550 84 mL of methanol for liquid chromatography.

Time span of measurement: For 30 minutes after injection,beginning after the pregabalin peak.

87 System suitability—

88 System performance: When the procedure is run with 20 89  $\mu$ L of the standard solution under the above operating condi-90 tions, the number of theoretical plates and the symmetry fac-91 tor of the peak of pregabalin are not less than 4000 and not 92 more than 1.8, respectively.

93 System repeatability: Pipet 5 mL of the standard solution,94 add the mobile phase to make exactly 100 mL. When the test

95 is repeated 6 times with 20  $\mu$ L of this solution under the 146 above operating conditions, the relative standard deviation of 147 96

97 the peak area of pregabalin is not more than 10%.

98 (iii) Total amount of related substances-The total of the 149 99 amount of related substances obtained in the Polar related substances and the Nonpolar related substances is not more 100 150 than 0.5%. 101

102 (2) Enantiomer—Dissolve 20 mg of Pregabalin in water 152 103 to make 10 mL. Transfer 500  $\mu$ L of this solution, 500  $\mu$ L of 104 a solution of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide in 105 acetonitrile (1 in 200) and 50  $\mu$ L of a solution of sodium hy-106 drogen carbonate (21 in 250) into a vial, stopper, shake, allow 107 to stand at 40°C for 1 hour, then add 50  $\mu$ L of 1 mol/L hydro-108 chloric acid TS and mix. To 400  $\mu$ L of this solution add 1600 109  $\mu$ L of the mobile phase, and use this solution as the sample solution. Perform the test with 20  $\mu$ L of the sample solution 159 110

as directed under Liquid Chromatography <2.01> according 111

to the following conditions. Determine the peak area,  $A_2$ , of 112

113 the pregabalin derivative in the sample solution and the peak

area, A1, of related substance B (enantiomer) derivative hav-114 115 ing the relative retention time of about 1.2 to the pregabalin

116 derivative by the automatic integral method:  $A_1 \swarrow (A_1 + A_2)$  is

not more than 0.0015. 117

118 Operating conditions—

119 Detector: An ultraviolet absorption photometer (wave-120 length: 340 nm).

121 Column: A stainless steel column 4.6 mm in inside diam-122 eter and 25 cm in length, packed with octadecylsilanized sil-

ica gel for liquid chromatography (5  $\mu$ m in particle diameter). 123

124 Column temperature: A constant temperature of about 125 30°C.

Mobile phase: To 620 mL of diluted triethylamine (1 in 126 127 100) adjusted to pH 3.0 with phosphoric acid add 380 mL of 128 acetonitrile for liquid chromatography.

129 Flow rate: Adjust so that the retention time of the pregabalin derivative is about 10 minutes. 130

System suitability-131

132 System performance: To 1 mL of the sample solution add 133 the mobile phase to make 100 mL and use this solution as the 134 solution for system suitability test. When the procedure is run 135 with 20  $\mu$ L of the solution for system suitability test under 136 the above operating conditions, the number of theoretical 137 plates and the symmetry factor of the peak of the pregabalin derivative are not less than 10,000 and not more than 1.5, re-138

139 spectively. 140 System repeatability: To 1 mL of the solution for system 141 suitability test add the mobile phase to make 20 mL. When the test is repeated 6 times with 20  $\mu$ L of this solution under 142

143 the above operating conditions, the relative standard devia-

144 tion of the peak area of the pregabalin derivative is not more

145 than 10%. Water <2.48>: Not more than 0.5% (0.2 g, coulometric titration).

#### 148 **Residue on ignition** <2.44>: Not more than 0.1% (2 g).

Assay Weigh accurately about 0.1 g each of Pregabalin and Pregabalin RS (separately determine the water <2.48> in the same manner as Pregabalin), dissolve in the mobile phase to make exactly 10 mL, respectively, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20  $\mu$ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas,  $A_T$  and  $A_S$ , of pregabalin in each solution.

Amount (mg) of pregabalin (C<sub>8</sub>H<sub>17</sub>NO<sub>2</sub>)= $M_S \times A_T / A_S$ 

M<sub>S</sub>: Amount (mg) of Pregabalin RS taken, calculated on the anhydrous basis

### **Operating** conditions—

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Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5  $\mu$ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.40 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 6.3 with ammonia solution (28). To 850 mL of this solution add 150 mL of methanol for liquid chromatography.

Flow rate: 1.0 mL per minute.

System suitability-

System performance: When the procedure is run with 20  $\mu$ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pregabalin are not less than 6600 and not more than 1.1, respectively.

System repeatability: When the test is repeated 6 times with 20  $\mu$ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pregabalin is not more than 1.0%.

184 Containers and storage Containers-Well-closed con-185 tainers.

#### 186 Others

187 Related substance A:

188 (4S)-4-(2-Methylpropyl)pyrrolidin-2-one



190 Related substance B (enantiomer): 191 (3R)-3-(Aminomethyl)-5-methylhexanoic acid

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193 Related substance C:

194 (2RS)-2-Hydroxy-2-phenylacetic acid

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CO<sub>2</sub>H and enantiomer

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- 196 Related substance D:
- 197 1-Methylethyl (2RS)-2-hydroxy-2-phenylacetate

$$\begin{array}{c} H & OH \\ \hline & O \\ \hline & O \\ O \\ CH_3 \\ \hline & O \\ CH_3 \\ \hline & and enantiomer \\ \end{array}$$

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199 Add the following to 9.01 Reference 200 Standards section (1):

- 201 Pregabalin RS
- 202 Add the following to 9.41 Reagents, Test 203 Solutions:

204 **1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide** 

- $205 \qquad C_9H_9FN_4O_5 \quad Light \ yellow \ or \ orange, \ crystals \ or \ powder.$
- 206 Melting point 226 229°C (beginning to melt)
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