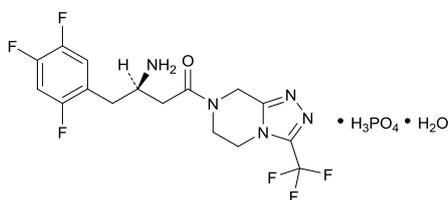


1 Sitagliptin Phosphate Hydrate

2 シタグリプチンリン酸塩水和物

3



4

5 $C_{16}H_{15}F_6N_5O \cdot H_3PO_4 \cdot H_2O$: 523.32

6 (3*R*)-3-Amino-1-[3-(trifluoromethyl)-5,6-

7 dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-

8 4-(2,4,5-trifluorophenyl)butan-1-one monophosphate monohydrate

9 [654671-77-9]

10

11 Sitagliptin Phosphate Hydrate contains not less
12 than 98.0% and not more than 102.0% of sitagliptin
13 phosphate ($C_{16}H_{15}F_6N_5O \cdot H_3PO_4$: 505.31), calculated
14 on the anhydrous basis.

15 **Description** Sitagliptin Phosphate Hydrate occurs as a
16 white powder.

17 It is soluble in water, sparingly soluble in methanol, very
18 slightly soluble in acetonitrile and in ethanol (99.5), and
19 practically insoluble in 2-propanol.

20 **Identification (1)** Determine the absorption spectrum
21 of a solution of Sitagliptin Phosphate Hydrate (1 in 10,000)
22 as directed under Ultraviolet-visible Spectrophotometry
23 <2.24>, and compare the spectrum with the Reference Spec-
24 trum or the spectrum of a solution of Sitagliptin Phosphate
25 RS prepared in the same manner as the sample solution:
26 both spectra exhibit similar intensities of absorption at the
27 same wavelengths.

28 **(2)** Determine the infrared absorption spectrum of
29 Sitagliptin Phosphate Hydrate as directed in the paste
30 method under Infrared Spectrophotometry <2.25>, and
31 compare the spectrum with the Reference Spectrum or the
32 spectrum of Sitagliptin Phosphate RS: both spectra exhibit
33 similar intensities of absorption at the same wave numbers.
34 Alternatively, perform the test by the potassium bromide
35 disk method or the ATR method, and compare the spectrum
36 with the spectrum of Sitagliptin Phosphate RS: both spectra
37 exhibit similar intensities of absorption at the same wave
38 numbers.

39 **(3)** A solution of Sitagliptin Phosphate Hydrate (1 in
40 25) responds to Qualitative Tests <1.09> (1) for phosphate.

41 **Purity (1)** Related substances—Use the sample solu-
42 tion obtained in the Assay as the sample solution. Pipet 1

43 mL of the sample solution, add a mixture of diluted phos-
44 phoric acid (1 in 1000) and acetonitrile for liquid chroma-
45 tography (19:1) to make exactly 1000 mL, and use this so-
46 lution as the standard solution. Perform the test with exactly
47 20 μ L each of the sample solution and standard solution as
48 directed under Liquid Chromatography <2.01> according to
49 the following conditions. Determine each peak area by the
50 automatic integration method: the area of the peak other
51 than sitagliptin obtained from the sample solution is not
52 larger than the peak area of sitagliptin from the standard
53 solution, and the total area of the peaks other than
54 sitagliptin from the sample solution is not larger than 5
55 times the peak area of sitagliptin from the standard solution.
56 For this calculation the peak area not larger than 1/2 times
57 the peak area of sitagliptin from the standard solution is ex-
58 cluded.

59 **Operating conditions** —

60 Detector, column, column temperature, mobile phase
61 and flow rate: Proceed as directed in the operating
62 conditions in the Assay.

63 Time span of measurement: About 5.5 times as long as
64 the retention time of sitagliptin.

65 **System suitability** —

66 System performance: Proceed as directed in the system
67 suitability in the Assay.

68 Test for required detectability: Pipet 5 mL of the
69 standard solution, and add a mixture of diluted phosphoric
70 acid (1 in 1000) and acetonitrile for liquid chromatography
71 (19:1) to make exactly 10 mL. Confirm that the SN ratio of
72 the peak of sitagliptin obtained with 20 μ L of this solution
73 is not less than 10.

74 System repeatability: When the test is repeated 6 times
75 with 20 μ L of the standard solution under the above
76 operating conditions, the relative standard deviation of the
77 peak area of sitagliptin is not more than 2.0%.

78 **(2) Enantiomer**—Dissolve 80 mg of Sitagliptin Phos-
79 phate Hydrate in a mixture of methanol and water (9:1) to
80 make 10 mL, and use this solution as the sample solution.
81 Perform the test with 10 μ L of the sample solution as di-
82 rected under Liquid Chromatography <2.01> according to
83 the following conditions. Determine the total peak area, A_T ,
84 of sitagliptin and related substance A (enantiomer), having
85 the relative retention time of about 0.9 to sitagliptin, and
86 the peak area, A_S , of related substance A (enantiomer), and
87 calculate the amount of the enantiomer by the following
88 equation: not more than 0.5%.

$$90 \quad \text{Amount (\% of enantiomer)} = A_S / A_T \times 100$$

91 **Operating conditions** —

92 Detector: An ultraviolet absorption photometer
(wavelength: 268 nm).

93 Column: A stainless steel column 4.6 mm in inside
94 diameter and 25 cm in length, packed with amylose tris-
95 (3,5-dimethylphenylcarbamate)-immobilized silica gel for
96 liquid chromatography (5 μm in particle diameter).

97 Column temperature: A constant temperature of about
98 35°C.

99 Mobile phase: A mixture of ethanol (99.5), heptane,
100 water and diethylamine (600:400:1:1).

101 Flow rate: 0.8 mL per minute.

102 *System suitability*—

103 Test for required detectability: Pipet 1 mL of the sample
104 solution, and dissolve in a mixture of methanol and water
105 (9:1) to make exactly 100 mL. Pipet 1 mL of this solution,
106 add a mixture of methanol and water (9:1) to make exactly
107 10 mL. Confirm that the SN ratio of the peak of sitagliptin
108 obtained with 10 μL of this solution is not less than 10.

109 System performance: Dissolve 8 mg of Sitagliptin
110 Phosphate RS for System Suitability in 1 mL of a mixture
111 of methanol and water (9:1). When the procedure is run
112 with 10 μL of this solution under the above operating
113 conditions, the resolution between the peak of related
114 substance A (enantiomer) and sitagliptin is not less than 1.5.

115 **Water** <2.48> 3.3 – 3.7% (0.3 g, volumetric titration, di-
116 rect titration).

117 **Residue on ignition** <2.44> Not more than 0.2% (1 g,
118 platinum crucible).

119 **Assay** Weigh accurately about 20 mg each of Sitagliptin
120 Phosphate Hydrate and Sitagliptin Phosphate RS (sepa-
121 rately determine the water <2.48> in the same manner as
122 Sitagliptin Phosphate Hydrate), dissolve each in a mixture
123 of diluted phosphoric acid (1 in 1000) and acetonitrile for
124 liquid chromatography (19:1) to make exactly 200 mL, and
125 use these solutions as the sample solution and the standard
126 solution, respectively. Perform the test with exactly 20 μL
127 each of the sample solution and standard solution as di-
128 rected under Liquid Chromatography <2.01> according to
129 the following conditions, and determine the peak areas, A_T
130 and A_S , of sitagliptin in each solution.

131 Amount (mg) of sitagliptin phosphate
132 ($\text{C}_{16}\text{H}_{15}\text{F}_6\text{N}_5\text{O}\cdot\text{H}_3\text{PO}_4$)
133 = $M_S \times A_T / A_S$

134 M_S : Amount (mg) of Sitagliptin Phosphate RS taken, cal-
135 culated on the anhydrous basis

136 *Operating conditions*—

137 Detector: An ultraviolet absorption photometer
138 (wavelength: 205 nm).

139 Column: A stainless steel column 4.6 mm in inside
140 diameter and 15 cm in length, packed with

141 cyanopropylsilylated silica gel for liquid chromatography
142 (5 μm in particle diameter).

143 Column temperature: A constant temperature of about
144 30°C.

145 Mobile phase: Dissolve 1.36 g of sodium dihydrogen
146 phosphate in 900 mL of water, adjust to pH 2.0 with
147 phosphoric acid, and add water to make 1000 mL. To 850
148 mL of this solution add 150 mL of acetonitrile for liquid
149 chromatography.

150 Flow rate: 1.0 mL per minute.

151 *System suitability*—

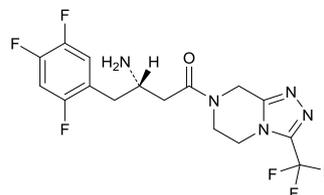
152 System performance: Place 10 mg of Sitagliptin
153 Phosphate RS and 1 mg of sodium stearyl fumarate in a vial,
154 and add 1 mL of water. Stopper the vial tightly, and heat at
155 80°C for 20 to 48 hours. Take out the contents of the vial,
156 wash the vial 3 times with a mixture of diluted phosphoric
157 acid (1 in 1000) and acetonitrile for liquid chromatography
158 (19:1), combine the washings and the content, and add a
159 mixture of diluted phosphoric acid (1 in 1000) and
160 acetonitrile for liquid chromatography (19:1) to make 100
161 mL. Stir this solution for 1 hour, and centrifuge for 10
162 minutes or until the solution becomes clear. Use the
163 supernatant liquid as the solution for system suitability test.
164 When the procedure is run with 20 μL of the solution for
165 system suitability test under the above operating conditions,
166 the resolution between sitagliptin and the peak having the
167 relative retention time of about 1.2 to sitagliptin is not less
168 than 1.5.

169 System repeatability: When the test is repeated 6 times
170 with 20 μL of the standard solution under the above
171 operating conditions, the relative standard deviation of the
172 peak area of sitagliptin is not more than 1.0%.

173 **Containers and storage** Containers—Tight containers.

174 **Others**

175 Related substance A (enantiomer): (3*S*)-3-Amino-1-[3-
176 (trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyra-
177 zine-7(8*H*)-yl]-4-(2,4,5-trifluorophenyl)butane-1-one



178

179 **Add the following to 9.01 Reference**
180 **Standards (1):**

181 **Sitagliptin Phosphate RS**

182 **Sitagliptin Phosphate RS for System Suitability**

183 *Add the following to 9.41 Reagents, Test*
184 *Solutions:*

185 **Sodium stearyl fumarate** $C_{22}H_{39}NaO_4$ A white crys-
186 talline powder.

187 *Content:* not less than 99.0% and not more than 101.5%,
188 calculated on the anhydrous basis. Assay — Weigh accu-
189 rately about 0.6 g of sodium stearyl fumarate, add 8 mL of
190 chloroform and 140 mL of acetic acid (100), and warm to
191 dissolve. After cooling, titrate <2.50> with 0.1 mol/L per-
192 chloric acid VS (potentiometric titration). Perform a blank
193 determination in the same manner, and make any necessary
194 correction.

195 Each mL of 0.1 mol/L perchloric acid VS
196 = 39.05 mg of $C_{22}H_{39}NaO_4$

197 *Add the following to 9.42 Solid Sup-*
198 *ports/Column Packings for Chromatog-*
199 *raphy:*

200 **Amylose tris-(3,5-dimethylphenylcarbamate)-immo-**
201 **bilized silica gel for liquid chromatography** Prepared
202 for liquid chromatography.
203