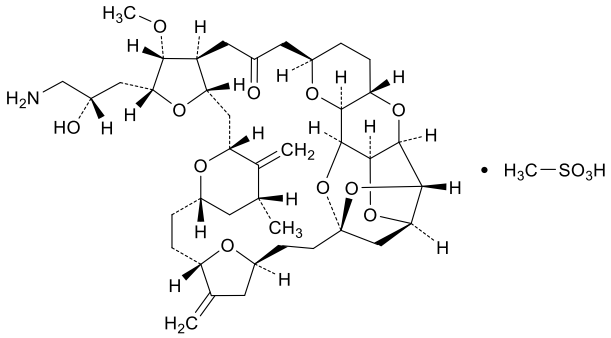
Eribulin Mesilate

エリブリンメシル酸塩



$C_{40}H_{59}NO_{11}.CH_4O_3S$: 826.00

(2*R*,3*R*,3a*S*,7*R*,8a*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29a*S*)-2-[(2*S*)-3-Amino-2-hydroxypropyl]-3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5(4*H*)-one monomethanesulfonate

[*441045-17-6*]

Eribulin Mesilate contains not less than 95.0% and not more than 102.0% of eribulin mesilate ($C_{40}H_{59}NO_{11}.CH_4O_3S$), and not less than 9.8% and not more than 12.2% of methanesulfonic acid, calculated on the anhydrous basis.

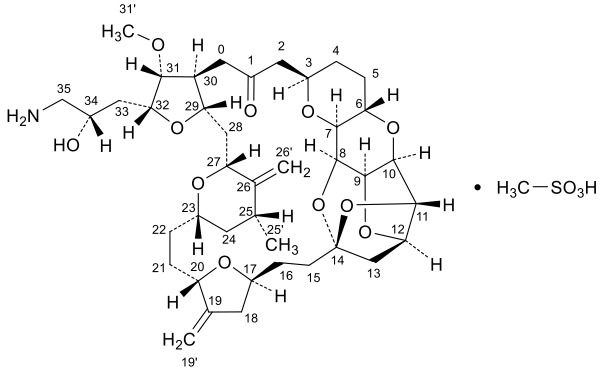
Manufacture Eribulin Mesilate has 19 chiral carbons, and its purity tests can not estimate all isomers derived from them. Therefore, based on sound science and the understanding of the product and the manufacturing process, control and manage the isomers and related substances during manufacturing process, and ensure the three-dimensional structure of eribulin mesilate. In the quality control strategy of Eribulin Mesilate, control the related substances including the principal isomers in the drug substance or starting materials and intermediates in upstream process. The acceptance value are not more than 0.22% and not more than 0.68% for the related substances B and C, which are the isomers (at position C34) and controlled in the drug substance, and are not more than the identification threshold (0.10%) for the related substances including other isomers. When Eribulin Mesilate is manufactured through the compounds 1 and 2, control as follows.

In the compound 1, control so that the isomers at positions C3 and C11, C12 (cis-olefin), and other related substances are not more than the identification threshold (0.10%). In the compound 2, control so that the isomers at positions C17 and C29 are not more than 0.30%, and the isomer at position C20 is not more than 0.50%, the isomer at position C25 is not more than 0.40%, and the isomers at positions C23, C27, C34 and C18/C19 (endo-olefin) and the other related substances are not more than the identification threshold (0.10%).

Furthermore, ensure that the isomers at positions C17, C20, C25 and C29 are not more than the identification threshold (0.10%) in the processes after the compounds 1 and 2, and the other related substances are not more than the qualification threshold (0.15%).

When manufactured without reaction using the compounds 1 and 2, perform the control based on the control mentioned above.

The position numbers of eribulin mesilate used in this Manufacture are as follows. The numbers are used commonly for the related substances, but are not related to the position numbers prescribed by the chemical names.



Position numbers of Eribulin Mesilate in Manufacture

Description Eribulin Mesilate occurs as a white powder.

It is freely soluble in water, in methanol, in ethanol (99.5) and in dimethylsulfoxide.

It is hygroscopic.

Identification (1) Determine the ^1H spectrum of a solution of Eribulin Mesilate in deuterated methanol for nuclear magnetic resonance spectroscopy (1 in 200), as directed under Nuclear Magnetic Resonance Spectroscopy *<2.21>*, using light hydrogen contaminated in deuterated methanol for nuclear magnetic resonance spectroscopy as an internal reference compound and the chemical shift of methyl group of deuterated methanol as δ 3.3 ppm: it exhibits a doublet signal A at around δ 1.1 ppm, a multiplet signal B and a singlet signal C at around δ 2.7 ppm, a singlet signal D at around δ 3.4 ppm, a doublet signal E at around δ 3.7 ppm, a doublet signal F at around δ 4.5 ppm, a triplet signal G at around δ 4.6 ppm, and a triplet signal H at around δ 4.7 ppm. The ratio of integrated intensity of these signals, A:B:C:D:E:F:G:H, is about 3:2:3:3:1:1:1:1. (Measure with a nuclear magnetic resonance spectrometer having ^1H resonance frequency of not less than 600 MHz.)

(2) Perform the test with 15 μL each of the sample solution and standard solution obtained in the Assay (2) as directed under Liquid Chromatography *<2.01>* according to the following conditions: the retention times of the peaks of methanesulfonic acid in the chromatograms obtained from the sample solution and standard solution are the same.

Operating conditions—

Proceed as directed in the operating conditions in the Assay (2).

System suitability—

Proceed as directed in the system suitability in the Assay (2).

Optical rotation <*2.49*> $[\alpha]_{365}^{20}$: −160 – −210° (50 mg calculated on the anhydrous and solvent-free basis, dimethylsulfoxide, 10 mL, 100 mm).

Purity **(1)** Heavy metals — Being specified separately when the drug is granted approval based on the Law.

(2) Related substances—Weigh accurately about 0.1 g of Eribulin Mesilate, dissolve in the dissolving solution to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Eribulin Mesilate RS (separately determine the water <*2.48*> in the same manner as Eribulin Mesilate), dissolve in the dissolving solution to make exactly 25 mL. Pipet 1 mL of this solution, add the dissolving solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions. Determine each peak area, A_T, of the related substances in the sample solution and the peak area, A_S, of eribulin in the standard solution by the automatic integration method, and calculate the amounts of the related substances by the following formula: the amounts of the related substance A having the relative retention time of about 0.29 to eribulin, the related substance B having the relative retention time of about 0.87, the related substance C having the relative retention time of about 1.07, the related substance D having the relative retention time of about 1.29, the related substance E having the relative retention time of about 1.37, and the related substance F having the relative retention time of about 1.67, are not more than 0.15%, 0.22%, 0.68%, 0.50%, 0.15%, and 0.19%, respectively, and other related substances are not more than 0.10%. Furthermore, the total amount of these related substances is not more than 3.0%.

$$\text{Amount (\%) of related substance} = M_S / M_T \times A_T / A_S$$

M_S: Amount (mg) of Eribulin Mesilate RS taken, calculated on the anhydrous basis

M_T: Amount (mg) of Eribulin Mesilate taken, calculated on the anhydrous basis

Dissolving solution—A mixture of water, acetonitrile for liquid chromatography and phosphoric acid (6500:3500:7) adjusted to pH 6.9 – 7.1 with diluted ammonium water (28) (1 in 5) or 1 mol/L hydrochloric acid TS.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1).

Time span of measurement: For 85 minutes after injection, beginning after the solvent peak.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay (1).

Test for required detectability: Pipet 1 mL of the standard solution, and add the dissolving solution to make exactly 20 mL. Confirm that the peak area of eribulin obtained with 5 μL of this solution is equivalent to 3.5 to 6.5% of that with 5 μL of the standard solution.

System repeatability: When the test is repeated 5 times with 5 μL of the solution for system suitability test obtained in the Assay (1) under the above operating conditions, the relative standard deviation of the peak area of eribulin is not more than 1.0%.

(3) Residual solvent—Being specified separately when the drug is granted approval based on the Law.

Water <*2.48*> Not more than 3.0% (Weigh accurately 30-50 mg of Eribulin Mesilate, dissolve in 5 mL of methanol for water determination, and perform the test with exactly 1 mL of this solution; coulometric titration).

Assay **(1)** Eribulin Mesilate—Weigh accurately about 0.1 g each of Eribulin Mesilate and Eribulin Mesilate RS, dissolve each in the dissolving solution to make exactly 25 mL, and use this solution as the sample solution and the standard solution. Perform the test with 5 μL 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 eribulin in each solution.

$$\text{Amount (mg) of eribulin mesilate } (C_{40}H_{59}NO_{11}.CH_4O_3S) = M_S \times A_T / A_S$$

M_S: Amount (mg) of Eribulin Mesilate RS taken, calculated on the anhydrous basis

Dissolving solution—A mixture of water, acetonitrile for liquid chromatography and phosphoric acid (6500:3500:7) adjusted to pH 6.9 – 7.1 with diluted ammonia water (28) (1 in 5) or 1 mol/L hydrochloric acid TS.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 200 nm).

Column: A stainless steel column 3 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 7.0 g of ammonium trifluoromethanesulfonate in 760 mL of water, add 3.0 mL of a solution of tetrabutylammonium dihydrogen phosphate (17

in 50) and 240 mL of acetonitrile for liquid chromatography, and adjust to pH 6.9 – 7.1 with diluted ammonium water (28) (1 in 5) or 1 mol/L hydrochloric acid TS.

Mobile phase B: Dissolve 7.0 g of ammonium trifluoromethanesulfonate in 300 mL of water, add 3.0 mL of a solution of tetrabutylammonium dihydrogen phosphate (17 in 50), 700 mL of acetonitrile for liquid chromatography and 20 mL of 2-propanol, and adjust to pH 6.9 – 7.1 with diluted ammonium water (28) (1 in 5) or 1 mol/L hydrochloric acid TS.

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 — 55	100	0
55 — 75	100 → 0	0 → 100
75 — 85	0	100
85 — 86	0 → 100	100 → 0
86 — 105	100	0

Flow rate: Control the flow rate as directed in the following table.

Time after injection of sample (min)	Flow rate (mL/minute)
0 – 55	0.50
55 – 75	0.50 → 0.63
75 – 105	0.63

System suitability —

System performance: Dissolve 2 mg of Eribulin Mesilate Related Substance C RS for system suitability in the dissolving solution to make 50 mL. To 1 mL of this solution add 20 mg of Eribulin Mesilate RS, dissolve in the dissolving solution to make 5 mL, and use this solution as the solution for system suitability test. When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, eribulin and the related substance C are eluted in this order with the resolution between these peaks being not less than 1.5. The number of theoretical plates and the symmetry factor of the peak of eribulin are not less than 13,500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 5 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of eribulin is not more than 1.0%.

(2) Methanesulfonic acid — Weigh accurately about 50 mg of Eribulin Mesilate, dissolve in a mixture of the mobile phase and acetonitrile for liquid chromatography (13:7) to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of methanesulfonic acid, dissolve in a mixture of the mobile phase and acetonitrile for liquid chromatography (13:7) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 15 μL 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 methanesulfonic acid in each solution. Calculate the content of methanesulfonic acid by the following formula.

$$\text{Content (\%) of methanesulfonic acid} = M_S \times A_T / A_S \times 10$$

M_S: Amount (mg) of methanesulfonic acid taken

Operating conditions —

Detector: An electric conductivity detector.

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

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.8 g of sodium dihydrogen phosphate monohydrate in 950 mL of water, add 11 μL of phosphoric acid, adjust to pH 4.2 – 4.3 with phosphoric acid if necessary, and add 50 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust so that the retention time of methanesulforic acid is about 6.5 minutes.

System suitability —

System performance: When the procedure is run with 15 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of methanesulforic acid are not less than 12,000 and not more than 0.7 – 1.5, respectively.

System repeatability: When the test is repeated 6 times with 15 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methanesulforic acid is not more than 3.0%.

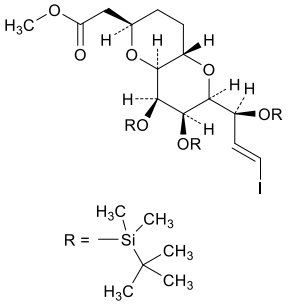
Containers and storage Containers — Tight containers.

Storage — Light-resistant, at a temperature not exceeding –65°C.

Others

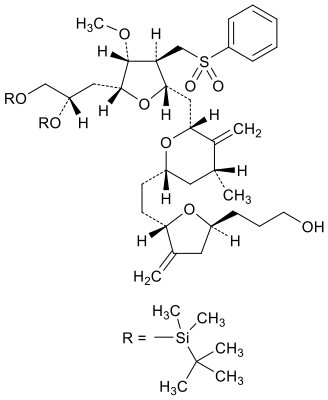
Compound 1:

Methyl {(2*R*,4a*S*,6*S*,7*R*,8*S*,8a*S*)-7,8-bis{[(1,1-dimethylethyl)dimethylsilyl]oxy}-6-[(1*S*,2*E*)-1-{[(1,1-dimethylethyl)dimethylsilyl]oxy}-3-iodoprop-2-en-1-yl]octahydropyrano[3,2-*b*]pyran-2-yl}acetate



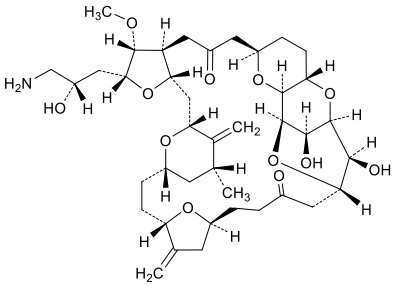
263

264 Compound 2:
265 3-[(2*S*,5*S*)-5-{2-[(2*S*,4*R*,6*R*)-6-({(2*S*,3*S*,4*R*,5*R*)-5-[(2*S*)-2,3-
266 Bis{[(1,1-dimethylethyl)dimethylsilyl]oxy}propyl]-4-
267 methoxy-3-[(phenylsulfonyl)methyl]tetrahydrofuran-
268 2-yl}methyl)-4-methyl-5-methylidenetetrahydro-2*H*-pyran-
269 2-yl]ethyl}-4- methylidenetetrahydrofuran-2-yl]propan-1-ol



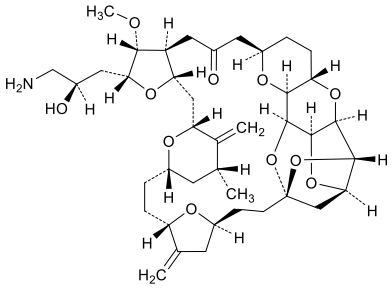
270

271 Related substance A:
272 (1*R*,3*S*,8*S*,11*S*,14*S*,16*R*,18*R*,20*S*,22*R*,23*R*,24*S*,28*R*,31*S*,33*S*,
273 34*R*,35*S*,37*S*)-22-[(2*S*)-3-Amino-2-hydroxypropyl]-34,37-
274 dihydroxy-23-methoxy-16-methyl-10,17-dimethylidene-
275 2,21,32,36,38,39-hexaoxaheptacyclo
276 [26.6.2.1^{3,33}.1^{8,11}.1^{14,18}.0^{20,24}.0^{31,35}]nonatriacontane-5,26-dione



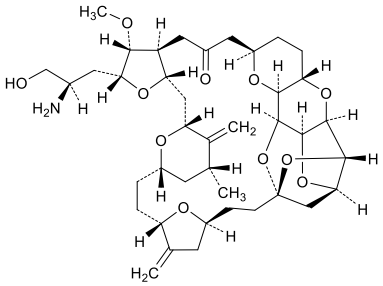
277

278 Related substance B:
279 (2*R*,3*R*,3a*S*,7*R*,8a*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,21*S*,
280 24*S*,26*R*,28*R*,29a*S*)-2-[(2*R*)-3-Amino-2-hydroxypropyl]-
281 3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-
282 11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-
283 9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]
284 dioxacyclopentacosin-5(4*H*)-one



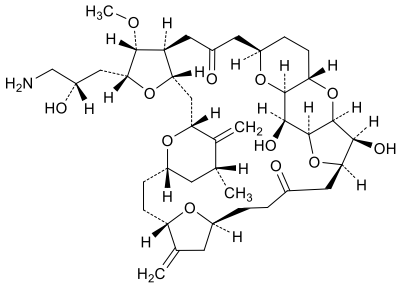
285

286 Related substance C:
287 (2*R*,3*R*,3a*S*,7*R*,8a*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,
288 21*S*,24*S*,26*R*,28*R*,29a*S*)-2-[(2*R*)-2-Amino-3-hydroxypropyl]-
289 3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-
290 11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-
291 9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]
292 dioxacyclopentacosin-5(4*H*)-one



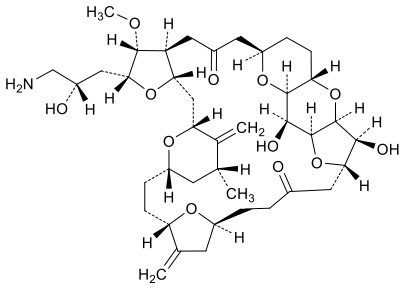
293

294 Related substance D:
295 (1*R*,2*S*,3*S*,4*S*,5*S*,6*R*,11*S*,14*S*,17*S*,19*R*,21*R*,23*S*,25*R*,26*R*,27*S*,
296 31*R*,34*S*)-25-[(2*S*)-3-Amino-2-hydroxypropyl]-2,5-
297 dihydroxy-26-methoxy-19-methyl-13,20-dimethylidene-
298 24,35,36,37,38,39-hexaoxaheptacyclo
299 [29.3.1.1^{3,6}.1^{4,34}.1^{11,14}.1^{17,21}.0^{23,27}]nonatriacontane-8,29-dione

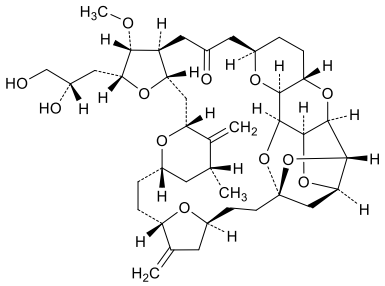


300

301 Related substance E:
302 (1*R*,2*S*,3*S*,4*S*,5*S*,6*S*,11*S*,14*S*,17*S*,19*R*,21*R*,23*S*,25*R*,26*R*,27*S*,
303 31*R*,34*S*)-25-[(2*S*)-3-Amino-2-hydroxypropyl]-2,5-
304 dihydroxy-26-methoxy-19-methyl-13,20-dimethylidene-
305 24,35,36,37,38,39-hexaoxaheptacyclo
306 [29.3.1.1^{3,6}.1^{4,34}.1^{11,14}.1^{17,21}.0^{23,27}]nonatriacontane-8,29-dione



Related substance F:
(2*R*,3*R*,3a*S*,7*R*,8a*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29a*S*)-2-[(2*S*)-2,3-Dihydroxypropyl]-3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5(4*H*)-one



Add the following to 9.01 Reference Standards (1):

Eribulin Mesilate RS

Eribulin Mesilate Related Substance C RS for System Suitability

Add the following to 9.41 Reagents, Test Solutions:

Ammoinium trifluoromethanesulfonate White, crystals or crystalline powder.

Identification－Determine the infrared absorption spectrum of ammoinium trifluoromethanesulfonate as directed in the ATR method under Infrared Spectrophotometry <*2.25*>: it exhibits absorption at the wave numbers of about 3190 cm^{-1}, 3090 cm^{-1}, 1227 cm^{-1}, 1164 cm^{-1}, and 1032 cm^{-1}.

Sodium dihydrogen phosphate monohydrate $NaH_2PO_4.H_2O$ White, crystals or crystalline powder. It is freely soluble in water, and practically insoluble in ethanol (99.5). It is slightly deliquescence with the atmospheric moisture.

pH <*2.54*>－The pH of a solution of 1.0 g of sodium dihydrogen phosphate monohydrate in 20 mL of water is between 4.1 and 4.5.

Change the following as follows:

Tetrabutylammonium dihydrogen phosphate
$(C_4H_9)_4NH_2PO_4$ White powder. It is soluble in water. For Eribulin Mesilate, when perform the test as directed in the system suitability in the Purity (2) under Eribulin Mesilate, the height of a peak appeared in the gradient mode is not more than 6 times the peak height of eribulin obtained from the standard solution.

Content: not less than 97.0%. Assay－Weigh accurately 1.5 g of tetrabutylammonium dihydrogen phosphate, dissolve in 80 mL of water, and titrate <*2.50*> with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS
＝169.7 mg of $(C_4H_9)_4NH_2PO_4$