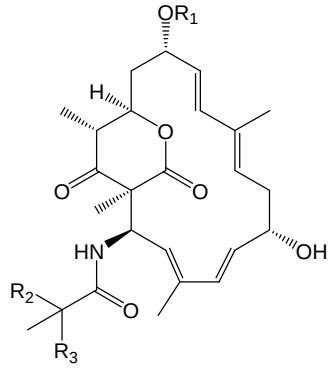
14 Sedecamycin

(Lankacidin)



Sedecamycin A	R_1: COCH3	R_2: =O	R_3: —	$C_{27}H_{35}NO_8$	MW: 501.6
Sedecamycin C	R_1: H	R_2: =O	R_3: —	$C_{25}H_{33}NO_7$	MW: 459.5
Sedecamycin D	R_1: COCH3	R_2: =H	R_3: OH	$C_{27}H_{37}NO_8$	MW: 503.6
Sedecamycin F	R_1: H	R_2: =H	R_3: OH	$C_{25}H_{35}NO_7$	MW: 461.6

CAS No.: 23477-98-7 (sedecamycin A)

[Summary of sedecamycin]

Sedecamycin (SCM) is a macrolactone antibiotic obtained by the incubation of *Streptomyces rochei var. volubilis*. SCM is composed of not less than 80% of sedecamycin A, 3 to 5% of sedecamycin C, 1 to 3% of sedecamycin D, and not more than 1% of sedecamycin F.

For physicochemical properties, SCM technical occurs as white to light red-yellow chrystals or christaline powder and has no odor or has a characteristic odor. It is freely soluble in acetonitrile and in chloroform, sparingly soluble in methanol and in anhydrous ethanol, and practically insoluble in water.

SCM has an antibacterial effect on Gram-positive bacteria and treponema bacteria and a growth promoting effect on pigs.

«Standards and specifications in the Act on Safety Assurance and Quality Improvement of Feeds, Guidelines for harmful substances, etc.»

SCM is a pure-grade antibiotic that was designated as a feed additive on June 22, 1993. The specifications for feeds containing this ingredient are specified in Appended Table 1, 1-(1)-C of the Ministerial Ordinance Concerning the Ingredient Specifications for Feeds and Feed Additives.

(in g(potency)/t)

Feed of interest	For pigs	
	Suckling piglets	Piglets
Added amount	5~20	5~20

The amount of SCM added to a commercial premix is roughly 2.5 to 10 g (potency)/kg.

[Methods listed in the Feed Analysis Standards]

1 Quantitative test methods - Plate method

1.1 Premix

[Feed Analysis Standards, Chapter 9, Section 2, 14.1.1]

A. Reagent preparation

1) Buffer solution: Buffer No.3

2) Esterolytic enzyme solution[1]. Place 5 mL of an esterolytic enzyme solution with a concentration of 3.5 units/mL[Note 1] in a 50-mL one-mark flask, add water up to the marked line, and filter through a membrane filter (pore size not exceeding 0.5 μm). Accurately diluthe the filtrate with water to prepare an esterolytic enzyme solution with a concentration of 0.035 unit/mL[2].

3) Sedecamycin standard solution. Weigh accurately not less than 40 mg of sedecamycin working standard[3], accurately add methanol and dissolve to prepare a sedecamycin standard stock solution with a concentration of 1 mg (potency)/mL[4].

At the time of use, accurately dilute a quantity of standard stock solution with Buffer No.3 to prepare high- and low-concentration standard solutions with concentrations of 2 and 0.5 μg (potency)/mL, respectively[5].

4) Culture medium: Medium F-4

5) Bacterial suspension and amount of addition. Use *Micrococcus luteus* ATCC 9341[6] as the test organism. Add about 0.2 mL of the suspension of the test organism per 100 mL of the culture medium, and add 1 mL of esterolytic enzyme solution per 100 mL of the culture medium.

6) Agar plate. Proceed by the agar well method.

B. Preparation of sample solution

Weigh accurately 3 to 5 g of the analysis sample, place in a 200-mL stoppered Erlenmeyer flask, add 100 mL of acetone, extract with stirring for 20 minutes, and filter the extract through filter paper (No.5A).

Accurately dilute a quantity of the filtrate with Buffer No.3 to prepare high- and low-concentration sample solutions with concentrations of 2 and 0.5 μg (potency)/mL, respectively[7].

C. Quantification [8]

Proceed by the 2-2 dose method[9].

Note 1. Esterolytic enzyme solution for quantification of sedecamycin (Wako Pure Chemical Industries, Ltd.) or an equivalent.

«Summary of analysis method»

This method is intended to determine the amount of SCM in a premix by microbiological assay using a sample solution prepared by extracting with acetone and diluting with Buffer No.3. The antibacterial effect on test organism *Micrococcus luteus* ATCC 9341 has been enhanced by adding an esteolytic enzyme solution to the culture medium to convert sedecamycin A into sedecamycin C. None of the antibacterial substances approved for combined use with SCM interfere with the quantification of SCM.

The flow sheet of this method is shown in Figure 9.2.14-1.

Sample (3.0-5.0 g)
↓
Extract with 100 mL of acetone.
(magnetic stirrer, 20 min)
↓
Filter (filter paper: No.5A).
↓
Dilute a quantity of the filtrate to prepare high- and low-concentration sample solutions (2 and 0.5 μg(potency)/mL, respectively).
↓
Dispense to agar plates (allow to stand at 10-20 °C for 2 hr).
↓
Incubate (at 35-37°C for 16-24 hr).
↓
Measure the inhibition zone diameter.
↓
Calculate the potency by the 2-2 dose method.

Figure 9.2.14-1 Quantitative test methods for sedecamycin (premix)

References: Takashi Sasaki, Yuzo Ono: Research Report of Animal Feed, 19, 135 (1994)

History in the Feed Analysis Standards [16] Addition

«Validation of analysis method»

・Spike recovery and repeatability

Sample type	Spike concentration (g(potency)/kg)	Repeat	Spike recovery (%)	Repeatability RSD(% or less)
Piglet premix 1	2.5~10	3	100.0~101.0	5.2
Piglet premix 2	2.5~10	3	96.7~102.3	8.3
Suckling piglet premix	2.5~10	3	96.0~103.3	4.9

・Collaborative study

No. of labs	Spike concentration (g(potency)/kg	Spike recovery (%)	Intra-lab repeatability RSDr(%)	Inter-lab reproducibility RSDR(%)	HorRat
7	5	103.9	2.2	4.9	1.10

«Notes and precautions»

[1] Addition of this enzyme solution to the culture medium is intended to convert sedecaymcin A into sedecamycin C. This conversion results in better quantification because of the greater antibacterial activity of sedecamycin C, which is 17 times greater than that of sedecamycin A.

Although sedecamycin D is also converted into sedecamycin F by the esterolytic enzyme, the antibacterial activity of sedecamycin F against *Micrococcus luteus* ATCC 9341 is about 1/6 times that of sedecamycin C.

[2] The instruments and equipments to be used shall have been sterilized and stored in a refrigerator.

[3] For the difinition etc. of sedecamycin working standard, refer to «Notes and precautions» [9] in Section 1, 1 of this Chapter.

[4] For the method of preparation for the standard stock solution, refer to «Notes and precautions» [10] in Section 1, 1 of this Chapter.

Method of preparation: example (when the weighed amount is 50 mg)

When the labeled potency of the working standard is 991 μg (potency)/mg, 50 mg of the working standard contains 49,550 μg (potency) (i.e., 50 mg × 991 μg (potency)/mg). To prepare a standard stock solution with a concentration of 1,000 μg (potency)/mL, the required amount of solvent is thus calculated to be 49.55 mL (i.e., 49,550 μg (potency) / 1,000 μg (potency)/mL). Therefore, completely transfer 50 mg of the working standard to an Erlenmeyer flask containing 49.55 mL of methanol, and dissolve to prepare a standard stock solution with a concentration of 1,000 μg (potency)/mL.

[5] For the method of preparation for the standard solution, refer to «Notes and precautions» [8] in Section 1, 1 of this Chapter.

An example method of preparation for sedecamycin standard solution is shown in Table 9.2.14-1.

[6] For the number of bacteria, refer to «Notes and precautions» [33] in Section 1, 1 of this Chapter.

[7] For the method of preparation for the sample solution, refer to «Notes and precautions» [8] in Section 1, 1 of this Chapter.

An example method of preparation is shown in Table 9.2.14-1.

Table 9.2.14-1 Method of preparation for sedecamycin standard solution and sample solution

1) Method of preparation for sedecamycin standard solution (premix, example)

Test tube No.	1	2	3	4
Amount (mL) of standard solution	②	2	4	5
Amount (mL) of Buffer No.3	18	18	16	15
Concentration (μg(potency)/mL)	100	10	2	0.5

Note: "② mL" means "2 mL of standard stock solution (1 mg(potency)/mL)".

2) Method of preparation for sample solution (premix, example)

When the analysis sample is collected in an amount equivalent to 20,000 μg (potency) of SCM, the concentration of SCM in the filtrate is calculated to be 200 μg (potency)/mL.

Test tube No.	1	2	3
Amount (mL) of sample solution	②	2	5
Amount (mL) of Buffer No.3	18	18	15
Concentraion (μg(potency)/mL)	20	2	0.5

Note "② mL" means "2 mL of the filtrate (200 μg(potency)/mL)".

[8] An example standard response line for SCM is shown in Figure 9.2.14-2.

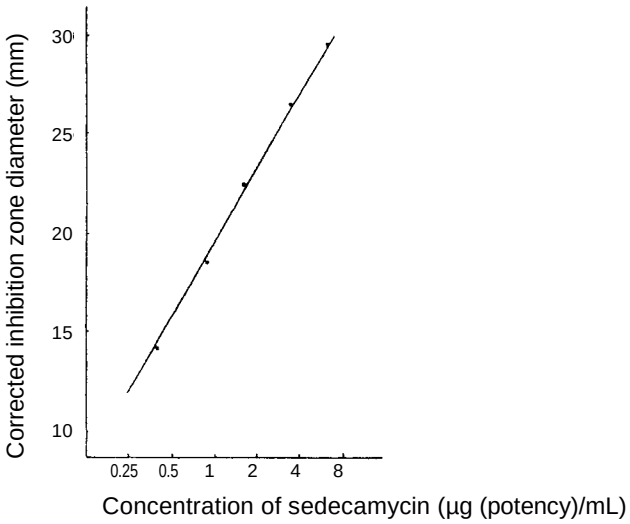


Figure 9.2.14-2 Standard response line for sedecamycin (premix, example)
(*Micrococcus luteus* ATCC 9341, Medium F-4, Agar well method)

[9] Refer to «Notes and precautions» [53] to [60] in Section 1, 1 of this Chapter.

1.2 Feeds, excluding heat-processed feeds, such as pellets

[Feed Analysis Standards, Chapter 9, Section 2, 14.2.1]

Scope of application: Feeds, excluding heat-processed feeds, such as pellets

A. Reagent preparation

1) Buffer solution: Buffer No.3
2) Dilution solvent: A mixture of Buffer No.3 and acetone (3:1)
3) Esterolytic enzyme solution. Place 5 mL of an esterolytic enzyme solution with a concentration of 3.5 units/mL in a 50-mL one-mark flask, add water up to the marked line, and filter through a membrane filter (pore diameter not exceeding 0.5 μm). Accurately dilute the filtrate with water to prepare an esterolytic enzyme solution with a concentration of 0.035 unit/mL.
4) Sedecamycin standard solution. Weigh accurately not less than 40 mg of sedecamycin working standard, accurately add methanol and dissolve to prepare a sedecamycin standard stock solution with a concentration of 1 mg (potency)/mL.

   At the time of use, accurately dilute a quantity of the standard stock solution with the dilution solvent to prepare standard solutions with concentration of 3.2, 1.6, 0.8, 0.4 and 0.2 μg (potency)/mL[1].
5) Culture medium: Medium F-4
6) Bacterial suspension and amount of addition. Use *Micrococcus luteus* ATCC 9341[2] as the test organism. Add about 0.2 mL of the suspension of the test organism per 100 mL of the culture medium, and add 1 mL of the esterolytic enzyme solution per 100 mL of the culture medium.
7) Agar plate. Proceed by the agar well method.

B. Preparation of sample solution

Extraction. Weigh accurately 4 to 16 g of the analysis sample (equivalent to 80 μg (potency) as SCM), place in a 200-mL stoppered Erlenmeyer flask, add 100 mL of ethyl acetate, extract with stirring for 20 minutes, and filter the extract through filter paper (No.5A). Transfer accurately 10 mL of the filtrate to a

50-mL round-bottom flask, condense alsomot into dryness under reduced pressure in a water bath at 50°C, and introduce nitrogen gas to evaporate into dryness.

Add 10 mL of dichloromethane to dissolve the residue, and use this solution as the sample solution subject to column treatment.

Column treatment. Wash a silica gel minicolumn (690 mg) with 10 mL of dichloromethane.

Transfer the sample solution to the minicolumn. Wash the round-bottom flask that contained the sample solution with three 2-mL portions of dichloromethane, and transfer the washings to the minicolumn. Inject the liquid under pressure to force out[Note 1][3]. Add 20 mL of a mixture of dichloromethane and ethyl acetate (97:3) to wash the minicolumn. Place a 50-mL round-bottom flask under the minicolumn, add 10 mL of ethyl acetate to the minicolumn, and inject under pressure[Note 1][3] to elute SCM.

Evaporate the eluate into dryness in a water bath at 50°C, add accurately 10 mL of the dilution solvent, and shake[4] to dissolve the residue to prepare a sample solution with a concentration of 0.8 μg (potency)/mL.

C. Quantification[5]

Proceed by the standard response line method[6].

Note 1. Set the flow rate to approximately 2 mL/min.

«Summary of analysis method»

This method is intended to determine the amount of SCM in an unheated feed, such as mash, by microbiological assay using a sample solution prepared by extracting with ethyl acetate and purifying through a silica gel minicolumn. None of the antibacterial substances approved for combined use with SCM interfere with the quantification of SCM.

The flow sheet of this method is shown in Figure 9.2.14-3.

Sample (4-16 g, equivalent to 80 μg(potency) as SCM)
↓
Extract with 100 mL of ethyl acetate.
(magnetic stirrer, 20 min)
↓
Filter (filter paper: No5A).
↓
Collect 10 mL of the filtrate (into a 50-mL round-bottom flask).
↓
Evaporate into dryness under reduced pressure (in a water bath at 50 °C).
↓
Dissolve the residue in 10 mL of dichloromethane.
↓
Load onto a silica gel minicolumn (previously washed with 10 mL of dichloromethane) (inject under pressure).
↓
Wash the round-bottom flask with 2 mL of dichloromethane and load on the silica gel minicolumn (repeat 3 times, inject under pressure).
↓
Wash the silica gel minicolumn with 20 mL of dichlormethane-ethyl acetate
↓
Elute SCM with 10 mL of ethyl acetate (into a 50-mL round-bottom flask, inject inder pressure).
↓
Evaporate into dryness under reduced pressure (in a water bath at 50°C).
↓
Dissolve the residue in 10 mL of Buffer No.3-acetone (3+1) (shake vigorously).
↓
Dispense to agar plates (allow to stand at 10-20°C for 2 hr).
↓
Incubate (at 35-37°C for 16-24 hr).
↓
Measure the inhibition zone diameter.
↓
Calculate the potency by the standard response line method.

Figure 9.2.14-3 Quantitative test method for sedecamycin (feed, exluding heat-processed feed, such as pellets)

References: Kiyoshi Kanno, Ayako Shirato: Research Report of Animal Feed, 18, 82 (1993)

History in the Feed Analysis Standards [15] New

«Validation of analysis method»

・Spike recovery and repeatability

Sample type	Spike concentration (g(potency)/t)	Repeat	Spike recovery (%)	Repeatability RSD(% or less)
Suckling piglet starter formula feed	5~20	3	99.4~105.5	3.4
Suckling piglet grower formula feed	5~20	3	100.2~107.8	8.7
Piglet formula feed	5~20	3	96.3~101.8	5.3

・Collaborative study

Sample type	No. of labs	Spike concentration (g(potency)/t)	Spike recovery (%)	Intra-lab repeatability RSDr(%)	Inter-lab reproducibility RSDR(%)
Suckling piglet grower formula feed	10	10	101.0	3.1	4.2

«Notes and precautions»

[1] For the method of preparation for the standard solution, refer to «Notes and precautions» [8] in Section 1, 1 of this Chapter.

An example method of preparation for the sedecamycin standard solution is shown in Table 9.2.14-2.

Table 9.2.14-2 Method of preparation for sedecamycin standard solution (feed, excluding heat-processed ones, such as pellets, example)

Test tube No.	1	2	3	4	5	6	7
Amount (mL) of standard solution	②	2	8	10	10	10	5
Amount (mL) of Buffer No.3-acetone (3:1)	23	18	12	10	10	10	5
Concentration (μg(potency)/ml	80	8	3.2	<1.6>	0.8	0.4	0.2

Note: ②mL" means "2 mL of standard stock solution (1 mg(potency)/mL)".

[2] For the number of bacteria, refer to «Notes and precautions» [33] in Section 1, 1 of this Chapter.

[3] Unforced flow is applicable if the liquid flows out smoothly.

[4] Although dissolution can usually be achieved by stoppering the flask and shaking by hand (for about 1 minute), it is recommended to apply ultrasonic waves.

[5] An example standard response line for SCM is shown in Figure 9.2.14-4.

Linearity is observed in the quantification range for SCM (SCM concentrations between 0.2 and 3.2 μg (potency)/mL).

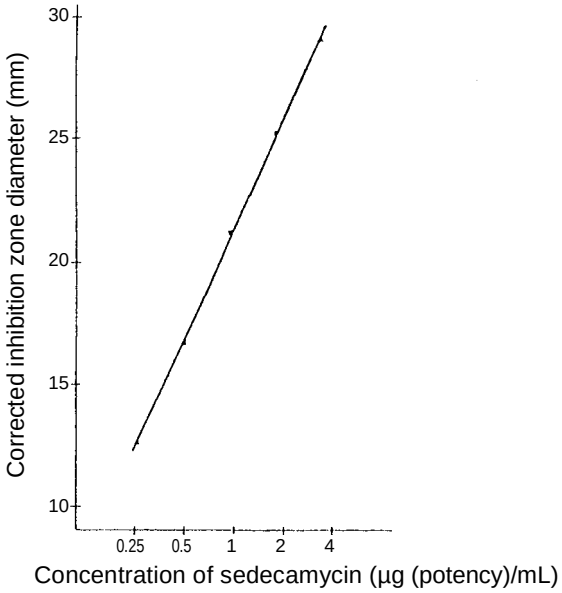


Figure 9.2.14-4 Standard response line for sedecamycin (unheated feed, example)
(*Micrococcus luteus* ATCC 9341, Medium F-4, Agar well method)

[6] Refer to «Notes and precautions» [53] to [57] and [61] in Section 1, 1 of this Chapter.

1.3 Heat-processed feed, such as pellets [Feed Analysis Standards, Chapter 9, Section 2, 14.2.2]

Scope of application: Heat-processed feed, such as pellets

A. Reagent preparation

1) Buffer solution: Buffer No.3
2) Buffer No.3 containing *β*-cyclodextrin. Dissolve 1.2 g of *β*-cyclodextrin in 1,000 mL of Buffer No.3, and sterilize by autoclaving at 121°C for 15 minutes.
3) Dilution solvent: A mixture of Buffer No.3 containing *β*-cyclodextrin and methanol (1:1)
4) Esterolytic enzyme solution. Place 5 mL of an esterolytic enzyme solution with a concentration of 3.5 units/mL in a 50-mL one-mark flask, add water up to the marked line, and filter through a membrane filter (pore size not exceeding 0.5 μm). Accurately dilute the filtrate with water to prepare an esterolytic enzyme solution with a concentration of 0.035 unit/mL.
5) Sedecamycin standard solution. Weigh accurately not less than 40 mg of sedecamycin working standard, accurately add methanol and dissolve to prepare a sedecamycin standard stock solution with a concentration of 1 mg (potency)/mL.

   At the time of use, accurately dilute a quantity of standard stock solution with the dilution solvent to prepare standard solutions with concentrations of 3.2, 1.6, 0.8, 0.4 and 0.2 μg (potency)/mL[1].
6) Culture medium: Medium F-4
7) Bacterial suspension and amount of addition. Use *Micrococcus luteus* ATCC 9341[2] as the test organism. Add about 0.2 mL of the suspension of the test organism per 100 mL of the culture medium, and add1 mL of the esterolytic enzyme solution per 100 mL of the culture medium.
8) Agar plate. Proceed by the agar well method.
9) Extracting solvent: A mixture of acetonitrile and water (4:1)

B. Preparation of sample solution

Extraction. Weigh accurately 4 to 16 g of the analysis sample (equivalent to 80 μg (potency) as SCM), place in a 200-mL stoppered Erlenmeyer flask, add 100 mL of the extracting solvent, extract with stirring for 20 minutes, and filter the extract through filter paper (No.5A). Transfer accurately 10 mL of the filtrate to a 200-mL stoppered Erlenmeyer flask, add 90 mL of water, shake, and use the solution as the sample solution subject to column treatment.

Column treatment. Connect two divinylbenzene-*N*-vinylpyrrolidone copolymer minicolumns (60 mg)[Note 1][3], and wash with 10 mL of methanol and 10 mL of water in this order.

Transfer the sample solution to the minicolumn, and inject under pressure to force out[Note 2][4]. Wash the Erlenmeyer flask that contained the sample solution with two 5-mL portions of water 2 times, and transfer the washings to the minicolumn. Inject the solution under pressure to force out[Note 2][4]. Add 10 mL of water to the minicolumn, and inject under pressure to wash the minicolumn[Note 2][4]. Place a 10-mL one-mark flask under the minicolumn, add 5 mL of methanol to the minicolumn, and inject under pressure to elute SCM[4]. Add Buffer No.3 containing *β*-cyclodextrin to the one-mark flask up to the marked line to prepare a sample solution with a concentration of 0.8 μg (potency)/mL.

C. Quantification[5]

Proceed by the standard response line method[6].

Note 1. Oasis HLB 3 cc (60 mg) Extraction Cartridge (Waters) connected to reservoir with a suitable capacity or an equivalent.
2. Set the flow rate to 2 to 3 mL/min.

«Summary of analysis method»

This method is intended to determine the amount of SCM in a heat-processed feed, such as pellets, by microbiological assay using a sample solution prepared by extracting with a mixture of acetonitrile and water (4:1) and purifying through a divinylbenzene-*N*-vinylpyrrolidone copolymer minicolumn. None of the antibacterial substances approved for combined use with SCM interfere with the quantification of SCM.

The flow sheet of this method is shown in Figure 9.14-5.

Sample (4-16 g, equivalent to 80 μg(potency) as SCM)
↓
Extract with 100 mL of acetonitrile-water (4:1).
(magnetic stirrer, 20 min)
↓
Filter (filter paper: No.5A).
↓
Add 90 mL of water to 10 mL of the filtrate (in a stoppered Erlenmeyer flask).
↓
Load onto a divinylbenzene-*N*-vinylpyrrolidone copolymer minicolumn (previously washed with 10 mL of methanol and 10 mL of water) (inject under
↓
Wash the stoppered Erlenmeyer flask with 5 mL of water and load on the divinylbenzene-N-vinylpyrrolidone copolymer minicolumn (repeat 2 times, inject under pressure).
↓
Wash the divinylbenzene-N-vinylpyrrolidone copolymer minicolumn with 10 mL of water.
↓
Elute SCM with 5 mL of methanol (into a 10-mL one mark flask, inject undre pr
↓
Add Buffer No.3 containing 0.12 % β-cyclodextrin to make 10 mL.
↓
Dispense to agar plates (allow to stand at 10-20°C for 2 hr).
↓
Incubate (at 35-37°C for 16-24 hr).
↓
Measure the inhibition zone diameter.
↓
Calculate the potency by the standard response line method.

Figure 9.14-5 Quantitative test method for sedecamycin (heat-processed feed, such as pellets)

References: Kiyoshi Kanno: Research Report of Animal Feed, 23, 118 (1998)

History in the Feed Analysis Standards [20] Addition

«Validation of analysis method»

・Spike recovery and repeatability

Sample type	Spike concentration (g(potency)/t)	Repeat	Spike recovery (%)	Repeatability RSD(% or less)
Suckling piglet formula feed	5~20	3	98.0~109.7	10.8
Piglet formula feed	5~20	3	96.3~105.0	7.6

・Collaborative study

Sample type	No. of labs	Spike concentration (g(potency)/t)	Spike recovery (%)	Intra-lab repeatability RSDr(%)	Inter-lab reproducibility RSDR(%)
Suckling piglet formula feed	8	10	98.8	2.8	5.6

«Notes and precautions»

[1] For the method of preparation for the standard solution, refer to «Notes and precautions» [8] in Section 1, 1 of this Chapter.

An example method of preparation for the sedecamycin standard solution is shown in Table 9.2.14-3.

Table 9.2.14-3 Method of preparation for sedecamycin standard solution (heat-processed feed, such as pellets, example)

Test tube No.	1	2	3	4	5	6	7
Amount (mL) of standard solution	②	2	8	10	10	10	5
Amount (mL) of Buffer No.3 containing 0.12 % β-cyclodextrin-methanol (1:1)	23	18	12	10	10	10	5
Concentration (μg(potency)/mL)	80	8	3.2	1.6	<0.8>	0.4	0.2

Note: "②mL" means "2 mL of standard stock solution (1 mg(potency)/mL)".

[2] For the number of bacteria, refer to «Notes and precautions» [33] in Section 1, 1 of this Chapter.

[3] The method for connecting the cartridges is shown in Figure 9.2.14-6.

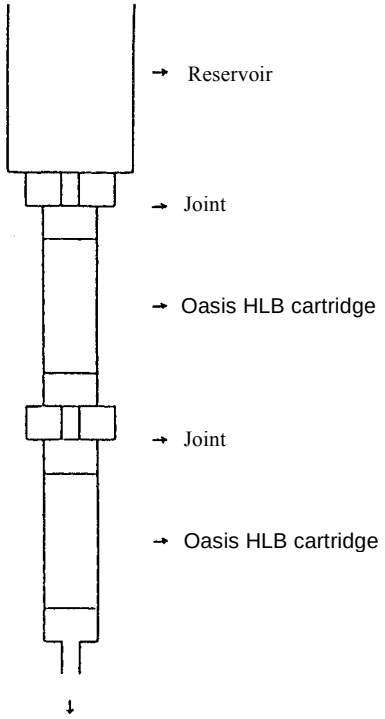


Figure 9.2.14-6 Method for connecting minicolumns

[4] To facilitate this procedure, it is advisable to use a vacuum manifold (Waters, etc.) in spite of injecting under pressure.

[5] An example standard response line for SCM is shown in Figure 9.2.14-7.

Linearity is observed in the quantification range for SCM (SCM concentrations between 0.2 and 3.2 μg (potency)/mL).

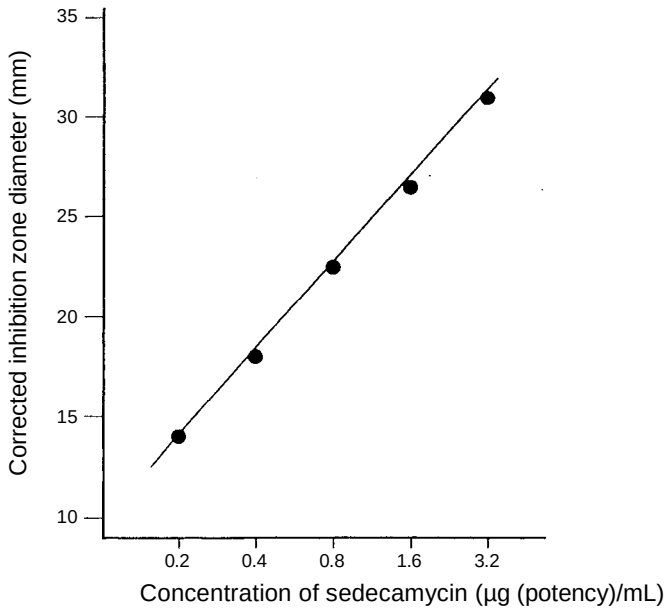


Figure 9.2.14-7 Standard response line for sedecamycin (heat-processed feed, such as pellets, example)

(*Micrococcus luteus* ATCC 9341, Medium F-4, Agar well method)

[6] Refer to «Notes and precautions» [53] to [57] and [61] in Section 1, 1 of this Chapter.