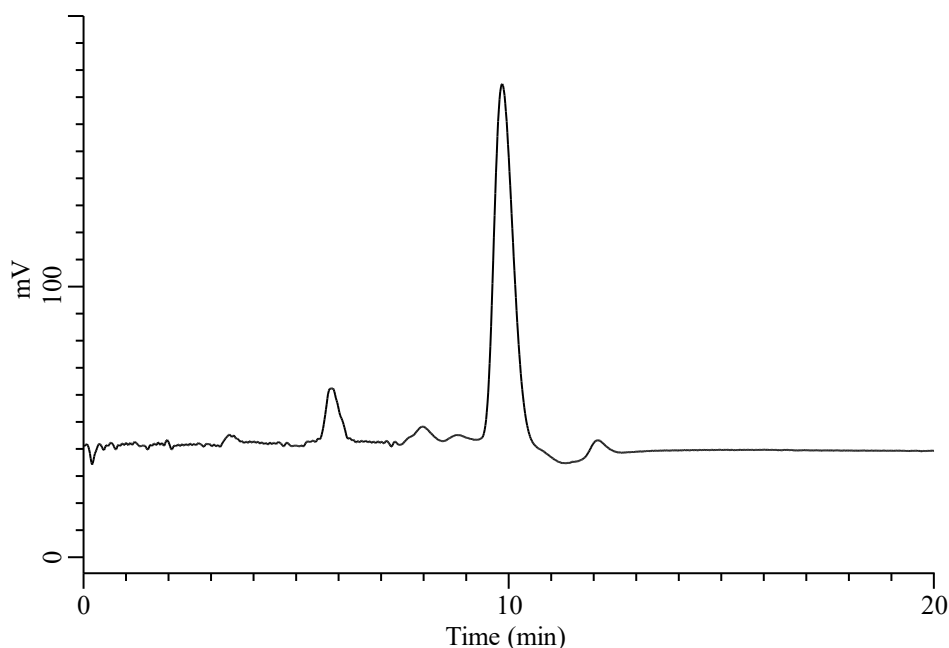


Analysis of Spectinomycin



Conditions

System : Chromaster PLUS (HITACHI)
Column : InertSustain C8 (GL Sciences Inc.)
 (5 μ m, 250 x 4.6 mm I.D.)
Column Cat. No. : 5020-16028
Eluent : A) CH₃CN
 B) Buffer *¹
 A/B = 1/10, v/v
Flow Rate : 1.0 mL/min
Reaction Reagent : 525 mM NaOH in H₂O
Reaction Flow Rate : 0.5 mL/min
Col. Temp. : 40 °C
Detection : ECD Pulse Mode (ED743, Gold)

Analyte:

Spectinomycin dihydrochloride pentahydrate
 0.8 mg/mL

E1:	120 mV	t1:	400 ms
E2:	700 mV	t2:	100 ms
E3:	-600 mV	t3:	100 ms
E4:	0 mV	t4:	0 ms
		ts:	50 ms

Injection Vol. : 20 μ L
Sample : Standard *²

*¹: Dissolve 4.2 g of oxalic acid and 2.0 mL of heptafluorobutyric acid in water and dilute to 1000 mL with water R; adjust to pH 3.2 with sodium hydroxide solution R, add 105 mL of acetonitrile R and mix; degas for 10 min.

*²: Dissolve 40.0 mg of spectinomycin hydrochloride (containing (4R)-dihydrospectinomycin) in water and dilute to 50.0 mL with the same solvent. Allow to stand for the same period of time as the test solution (formation of anomers). Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.