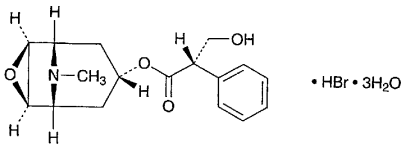
(100) and 30 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.04 mg of $C_{21}H_{30}BrNO_4$

Containers and storage Containers—Tight containers.

Scopolamine Hydrobromide

臭化水素酸スコポラミン



$C_{17}H_{21}NO_4.HBr.3H_2O$: 438.31
(1*S*,2*S*,4*R*,5*R*,7*s*)-9-Methyl-3-oxa-9-azatricyclo-[3.3.1.$0^{2,4}$]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate monohydrobromide trihydrate [*6533-68-2*]

Scopolamine Hydrobromide, when dried, contains not less than 98.5% of $C_{17}H_{21}NO_4.HBr$ (mol. wt.: 384.26).

Description Scopolamine Hydrobromide occurs as colorless or white crystals, or white granules or powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

Identification (1) To 1 mg of Scopolamine Hydrobromide add 3 to 4 drops of fuming nitric acid, evaporate on a water bath to dryness, and cool. Dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced.

(2) A solution of Scopolamine Hydrobromide (1 in 20) responds to the Qualitative Tests for bromide.

Optical rotation $[\alpha]_D^{20}$: −24.0 – −26.0° (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point 195 – 199°C (after drying; previously heat the bath to 180°C).

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Scopolamine Hydrobromide in 10 mL of water: the solution is clear and colorless.

(2) Acid—Dissolve 0.50 g of Scopolamine Hydrobromide in 15 mL of water, and add 0.50 mL of 0.02 mol/L sodium hydroxide and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) Apoatropine—Dissolve 0.20 g of Scopolamine Hydrobromide in 20 mL of water, add 0.60 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color in the solution does not disappear.

(4) Other alkaloids—Dissolve 0.15 g of Scopolamine Hydrobromide in 3 mL of water, and use this solution as the sample solution.

(i) To 1 mL of the sample solution add 2 to 3 drops of ammonia TS: no turbidity is produced.

(ii) To 1 mL of the sample solution add 2 to 3 drops of potassium hydroxide TS: a transient white turbidity might be produced, and disappears clearly in a little while.

Loss on drying Not more than 13.0% [1.5 g; first dry in a desiccator (silica gel) for 24 hours, then dry at 105°C for 3 hours].

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Scopolamine Hydrobromide, previously dried in 10 mL of acetic acid (100) by warming. After cooling, add 40 mL of acetic anhydride, and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.427 mg of $C_{17}H_{21}NO_4.HBr$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Secretin

セクレチン

His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2

$C_{130}H_{220}N_{44}O_{41}$: 3055.41
[*1393-25-5*]

Secretin is a peptide obtained from the upper part of hog small intestine (duodenum mucous membrane), having a pancreatic juice secretion-stimulating activity. It contains not less than 16,000 secretin Units and not more than 21,500 secretin Units per 1 mg, calculated on the de-acetic acid basis.

Description Secretin occurs as a white to pale yellow-white powder.

Identification Dissolve an amount of Secretin in bovine serum albumin TS for secretin so that each mL of the solution contains 20 secretin Units, and use this solution as the sample solution. Separately, dissolve an amount of Secretin Reference Standard in bovine serum albumin TS for Secretin Reference Standard so that each mL of the solution contains 20 secretin Units, and use this solution as the standard solution. Anesthetize a male Wistar rat, weighing 300 to 400 g, starved in advance for 24 hours, by injecting 1.2 g per kg body mass of ethyl carbamate into the abdominal cavity. Fix the animal on the back, cut and open the skin to reveal the femoral vein, insert a cannula filled with isotonic sodium chloride solution into the vein, and sew up the incision. Through the cannula inject 0.2 mL of the standard solution. Shave off the fur of the abdominal region, cut and open the region 3 to 4 cm below the central xiphoid process, and tie up the common bile duct at the duodenal ostial and the stomach at the pylorus, then insert a cannula into an upper

part of the common bile duct to make an artificial pancreatic duct. Separately, insert one end of a cannula into the common bile duct at the liver side and insert the other end of the cannula into the duodenum to allow the secretion of bile. Sew up the incision after settling the cannula so that the pancreatic duct is not distorted. Inject 0.2 mL of the standard solution through the femoral vein cannula to stabilize the sensitivity to secretin of the animal as well as to confirm the secretion of pancreatic juice. Maintain the intestinal temperature of the animal between 37°C and 38°C during the test. About 35 minutes later, inject 25 μL of the sample solution through the femoral vein cannula, and determine the amount of pancreatic juice secreted from the artificial pancreatic duct by using a glass tube with μL graduations: the ratio, S_{30}/S_B, of the amount secreted for 30 minutes after the injection of the sample solution (S_{30}) to the total of the amount secreted for 15 minutes before the injection and the amount secreted between 30 minutes and 45 minutes after the injection (S_B) is not less than 1.5. If the ratio is less than 1.5, repeat the test 3 times: at least 2 results so obtained are not less than 1.5.

Purity **(1)** 3-β-Aspartylsecretin—To 0.2 mg of Secretin add 0.20 g of L-cysteine hydrochloride monohydrate, and dissolve in 20 mL of a mixture of a solution of perchloric acid (1 in 100) and acetonitrile (7:3). Perform the test with 100 μL of this solution as directed under the Liquid Chromatography according to the following conditions, and calculate the peak areas, Aa and Ab, of secretin and 3-β-aspartylsecretin by the automatic integration method: $A\text{b}/(A\text{a}+A\text{b})$ is not more than 0.2.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions of the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of secretin from 100 μL of the standard solution obtained in the Assay is between 5 cm and 10 cm.

(2) Amino acids—Dissolve exactly 0.2 mg of Secretin in exactly 0.1 mL of water, and use this solution as the sample solution. Separately, dissolve exactly 1.0 mg of glycine in exactly 100 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100) and water (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution prepared by dissolving 1 g of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) to make 100 mL, heat the plate at 80°C for 10 minutes: no spot other than the principal spot from the sample solution is more intense than the spot from the standard solution.

(3) Acetic acid—Weigh accurately about 1.5 mg of Secretin, put it in a test tube about 1 cm in diameter and about 10 cm in length, add exactly 0.5 mL of a solution of *p*-toluenesulfonic acid monohydrate (1 in 200), seal the tube, and heat at 90–95°C in a water bath for 1 hour. After cooling, open the tube, and use the liquid content as the sample solution. Separately, pipet 10 μL of acetic acid (100), add a solution of *p*-toluenesulfonic acid monohydrate (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and the standard solution as directed under the Gas Chromatography according to the following conditions, and determine the peak heights, H_T and H_S, of acetic acid of these solutions. Calculate the amount of acetic acid by use of the following equation: not more than 5%.

$$\text{Amount (\%) of acetic acid } (C_2H_4O_2) = \frac{H_T}{H_S} \times 1.05 \times \frac{5}{\text{amount (mg) of sample}}$$

1.05: Specific gravity of acetic acid (100)

Operating conditions—

Detector: A hydrogen flame-ionization detector

Column: A glass column 3–4 mm in inside diameter and about 2 m in length, packed with porous polymer beads for gas chromatography (180–250 μm in particle diameter).

Column temperature: A constant temperature of about 200°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of acetic acid is 4–6 minutes.

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions, and calculate the number of theoretical plates (n) of the acetic acid peak. Use a column giving a value of n of not less than 500.

Assay Weigh accurately about 0.2 mg of Secretin, add 0.20 g of L-cysteine hydrochloride monohydrate, dissolve in exactly 20 mL of a mixture of a solution of perchloric acid (1 in 100) and acetonitrile (7:3), and use this solution as the sample solution. Separately, to the whole amount of Secretin Reference Standard in one ampoule add exactly 0.50 mL of a mixture of a solution of perchloric acid (1 in 100) and acetonitrile (7:3) to dissolve it, and use this solution as the standard solution. Perform the test with 100 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following operating conditions, and determine peak areas, A_T and A_S, of secretin of these solutions.

$$\text{Amount (Units) of secretin per mg of Secretin, calculated on the de-acetic acid basis} = \frac{A_T}{A_S} \times \frac{U_S}{W_T} \times 40 \times \frac{100}{100 - L}$$

U_S: Amount of secretin (Units) in one ampoule of Secretin Reference Standard

W_T: Amount (mg) of sample

L: Amount (%) of acetic acid

Operating conditions—

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

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

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

Mobile phase: A mixture of 0.2 mol/L sodium perchlorate solution and acetonitrile (3:2) adjusted to pH 3.0 with 1 mol/L phosphoric acid solution.

Flow rate: Adjust the flow rate so that the retention time of secretin is about 8 minutes.

Selection of column: Proceed with 100 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column from which 3-β-aspartylsecretin and secretin are eluted in this order with the resolu-

tion between these peaks being not less than 1.2.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of secretin is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Preserve at −20°C or lower.

Silver Nitrate

硝酸銀

$AgNO_3$: 169.87

Silver Nitrate, when dried, contains not less than 99.8% of $AgNO_3$.

Description Silver Nitrate occurs as lustrous, colorless or white crystals.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually turns grayish black by light.

Identification A solution of Silver Nitrate (1 in 50) responds to the Qualitative Tests for silver salt and for nitrate.

Purity **(1)** Clarity and color of solution, and acidity or alkalinity—Dissolve 1.0 g of Silver Nitrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless. It is neutral.

(2) Bismuth, copper and lead—To 5 mL of a solution of Silver Nitrate (1 in 10) add 3 mL of ammonia TS: the solution is clear and colorless.

Loss on drying Not more than 0.20% (2 g, silica gel, light resistant, 4 hours).

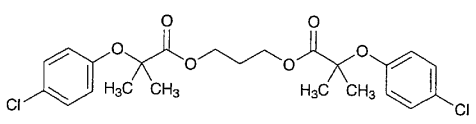
Assay Weigh accurately about 0.7 g of Silver Nitrate, previously powdered and dried, dissolve in 50 mL of water, add 2 mL of nitric acid, and titrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 16.987 mg of $AgNO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Simfibrate

シンフィブラート



$C_{23}H_{26}Cl_2O_6$: 469.35
Trimethylene bis[2-(4-chlorophenoxy)-2-methylpropanoate]
[*14929-11-4*]

Simfibrate, when dried, contains not less than 98.5% of $C_{23}H_{26}Cl_2O_6$.

Description Simfibrate occurs as white to light yellow crystals or crystalline powder. It is odorless and tasteless.

It is very soluble in acetonitrile and in diethyl ether, soluble in ethanol (95) and in hexane, and practically insoluble in water.

Identification **(1)** To 0.05 g of Simfibrate add 0.5 mL of ethanol (95), and dissolve by warming on a water bath. After cooling, add 0.3 mL of a saturated solution of hydroxylammonium chloride in ethanol (95) and 0.3 mL of potassium hydroxide-ethanol TS, heat gently to boiling, and cool. To this solution add 1 mL of 1 mol/L hydrochloric acid TS and 2 mL of ethanol (95), and then add 1 drop of iron (III) chloride TS: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Simfibrate in hexane for ultraviolet-visible spectrophotometry (3 in 50,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Simfibrate in hexane for ultraviolet-visible spectrophotometry (3 in 200,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Simfibrate as directed under the Flame Coloration Test (2): a green color appears.

Melting point 49 - 53°C

Purity **(1)** Clarity and color of solution—Dissolve 1.0 g of Simfibrate in 10 mL of ethanol (95) by warming on a water bath: the solution is clear and colorless to light yellow.

(2) Acid—To 4.0 g of Simfibrate add 40 mL of neutralized ethanol, dissolve by warming on a water bath, cool, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals—Proceed with 1.0 g of Simfibrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic—Prepare the test solution with 1.0 g of Simfibrate according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(5) *p*-Chlorophenol—To 2.0 g of Simfibrate add exactly 1 mL of the internal standard solution, dissolve in acetonitrile to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.10 g of 4-chlorophenol in acetonitrile to make exactly 100 mL. Pipet 2 mL of this solution, and add acetonitrile to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution and acetonitrile to make 10 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Calculate the ratios, Q_T and Q_S, of the peak height of 4-chlorophenol to that of the internal standard from each solution: Q_T is not larger than Q_S.

Internal standard solution—A solution of 4-ethoxyphenol in acetonitrile (1 in 50,000).