

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* (1).

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Bacterial endotoxins (85)—It contains not more than 3.88 USP Endotoxin Units per mg of anhydrous pamidronate disodium.

Uniformity of dosage units (905): meets the requirements for *Weight Variation*.

pH (791): between 6.0 and 7.0, determined in a solution constituted as directed in the labeling.

Particulate matter (788): meets the requirements for small-volume injections.

Water, Method 1a (921): not more than 5%.

Limit of beta alanine—

Adsorbent, Application volume, Developing solvent system, and Spray reagent—Proceed as directed for *Related compounds, Test 1* under *Pamidronate Disodium*.

Standard solution—Dissolve an accurately weighed quantity of USP Beta Alanine RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution containing 0.0075 mg of beta alanine per mL.

Test solution—Reconstitute the vial with the appropriate amount of water to obtain a solution having a concentration of 3 mg of anhydrous pamidronate disodium per mL, based on the label claim.

Procedure— Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 minutes. Examine the plate under white light. The spot having an *R_f* value of about 0.5 obtained from the *Test solution* is not greater in size or intensity than the corresponding spot obtained from the *Standard solution*: not more than 0.25% of beta alanine is found.

Other requirements—It meets the requirements under *Sterility Tests* (71) and for *Labeling* under *Injections* (1).

Assay—

Mobile phase and Chromatographic system—Proceed as directed in the *Assay* under *Pamidronate Disodium*.

Standard preparation—Dissolve an accurately weighed quantity of USP Pamidronate Disodium RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 2.5 mg per mL. Calculate the concentration, *C_s*, of anhydrous pamidronate disodium, the molecular weights of anhydrous and pentahydrate pamidronate disodium being 279.06 and 369.11, respectively.

Assay preparation—Constitute a suitable number of vials of Pamidronate Disodium for *Injection* with the appropriate amount of water to obtain a solution having a known concentration of about 2 mg of anhydrous pamidronate disodium per mL, based on the label claim.

Procedure—Separately inject equal volumes (about 100 μ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of $C_3H_9NNa_2O_7P_2$ in the portion of Pamidronate Disodium for *Injection* taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which *C_s* is as defined under the *Standard preparation*; *C_u* is the concentration, in mg per mL, of anhydrous pamidronate disodium in the *Assay preparation*; and *r_u* and *r_s* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pancreatin

Pancreatin.

Pancreatin [8049-47-6].

» Pancreatin is a substance containing enzymes, principally amylase, lipase, and protease, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae) or of the ox, *Bos taurus* Linné (Fam. Bovidae). Pancreatin contains, in each mg, not less than 25 USP Units of amylase activity, not less than 2.0 USP Units of lipase activity, and not less than 25 USP Units of protease activity. Pancreatin of a higher digestive power may be labeled as a whole-number multiple of the three minimum activities or may be diluted by admixture with lactose, or with sucrose containing not more than 3.25 percent of starch, or with pancreatin of lower digestive power.

NOTE—One USP Unit of amylase activity is contained in the amount of pancreatin that decomposes starch at an initial rate such that 0.16 μ Eq of glycosidic linkage is hydrolyzed per minute under the conditions of the *Assay for amylase activity*.

One USP Unit of lipase activity is contained in the amount of pancreatin that liberates 1.0 μ Eq of acid per minute at a pH of 9.0 and 37° under the conditions of the *Assay for lipase activity*.

One USP Unit of protease activity is contained in the amount of pancreatin that under the conditions of the *Assay for protease activity* hydrolyzes casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the same absorbance at 280 nm as 15 nmol of tyrosine.

Packaging and storage—Preserve in tight containers, at a temperature not exceeding 30°.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

Microbial enumeration tests (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the test for absence of *Salmonella* species and *Escherichia coli*.

Loss on drying (731)—Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Fat—Place 2.0 g of Pancreatin in a flask of about 50-mL capacity, add 20 mL of ether, insert the stopper, and set it aside for 2 hours, mixing by rotating at frequent intervals. Decant the supernatant ether by means of a guiding rod into a plain filter about 7 cm in diameter, previously moistened with ether, and collect the filtrate in a tared beaker. Repeat the extraction with a 10-mL portion of ether, proceeding as directed before, then with another 10-mL portion of ether, and transfer the ether and the remainder of the Pancreatin to the filter. Allow to drain, evaporate the ether spontaneously, and dry the residue at 105° for 2 hours: the residue of fat obtained from Pancreatin possessing three or more times the three minimum activities weighs not more than 120 mg (6.0%); that obtained from Pancreatin possessing less than three times the three minimum activities weighs not more than 60 mg (3.0%).

Assay for amylase activity (Starch digestive power)—

pH 6.8 phosphate buffer—On the day of use, dissolve 13.6 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 14.2 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 51 mL of the monobasic potassium phosphate solution with 49 mL of the dibasic sodium phosphate solution. If necessary, adjust by the dropwise addition of the appropriate solution to a pH of 6.8.

Substrate solution—On the day of use, stir a portion of purified soluble starch equivalent to 2.0 g of dried substance with 10 mL of water, and add this mixture to 160 mL of boiling water. Rinse the beaker with 10 mL of water, add it to the hot solution, and heat to boiling, with continuous mixing. Cool to room temperature, and add water to make 200 mL.

Standard preparation—Weigh accurately about 20 mg of USP Pancreatin Amylase and Protease RS into a suitable mortar. Add about 30 mL of *pH 6.8 phosphate buffer*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 phosphate buffer* to a 50-mL volumetric flask, dilute with *pH 6.8 phosphate buffer* to volume, and mix. Calculate the activity, in USP Units of amylase activity per mL, of the resulting solution from the declared potency on the label of the USP Reference Standard.

Assay preparation—For Pancreatin having about the same amylase activity as the USP Pancreatin Amylase and Protease RS, weigh accurately about 40 mg of Pancreatin into a suitable mortar. [NOTE—For Pancreatin having a different amylase activity, weigh accurately the amount necessary to obtain an *Assay preparation* having amylase activity per mL corresponding approximately to that of the *Standard preparation*.] Add about 3 mL of *pH 6.8 phosphate buffer*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *pH 6.8 phosphate buffer* to a 100-mL volumetric flask, dilute with *pH 6.8 phosphate buffer* to volume, and mix.

Procedure—Prepare four stoppered, 250-mL conical flasks, and mark them *S*, *U*, *BS*, and *BU*. Pipet into each flask 25 mL of *Substrate solution*, 10 mL of *pH 6.8 phosphate buffer*, and 1 mL of sodium chloride solution (11.7 in 1000), insert the stoppers, and mix. Place the flasks in a water bath maintained at $25 \pm 0.1^\circ$, and allow them to equilibrate. To flasks *BU* and *BS* add 2 mL of 1 N hydrochloric acid, mix, and return the flasks to the water bath. To flasks *U* and *BU* add 1.0-mL portions of the *Assay preparation*, and to flasks *S* and *BS* add 1.0 mL of the *Standard preparation*. Mix each, and return the flasks to the water bath. After 10 minutes, accurately timed from the addition of the enzyme, add 2-mL portions of 1 N hydrochloric acid to flasks *S* and *U*, and mix. To each flask, with continuous stirring, add 10.0 mL of 0.1 N iodine VS, and immediately add 45 mL of 0.1 N sodium hydroxide. Place the flasks in the dark at a temperature between 15° and 25° for 15 minutes. To each flask add 4 mL of 2 N sulfuric acid, and titrate with 0.1 N sodium thiosulfate VS to the disappearance of the blue color. Calculate the amylase activity, in USP Units per mg, of the Pancreatin taken by the formula:

$$100(C_S / W_U)(V_{BU} - V_U) / (V_{BS} - V_S)$$

in which C_S is the amylase activity of the *Standard preparation*, in USP Units per mL, W_U is the amount, in mg, of Pancreatin taken, and V_U , V_S , V_{BU} , and V_{BS} are the volumes, in mL, of 0.1 N sodium thiosulfate consumed in the titration of the solutions in flasks *U*, *S*, *BU*, and *BS*, respectively.

Assay for lipase activity (Fat digestive power)—

Acacia solution—Centrifuge a solution of acacia (1 in 10) until clear. Use only the clear solution.

Olive oil substrate—Combine 165 mL of *Acacia solution*, 20 mL of olive oil, and 15 g of crushed ice in the cup of an electric blender. Cool the mixture in an ice bath to 5° , and homogenize at high speed for 15 minutes, intermittently cooling in an ice bath to prevent the temperature from exceeding 30° .

Test for suitability of mixing as follows. Place a drop of the homogenate on a microscope slide, and gently press a cover slide in place to spread the liquid. Examine the entire field

under high power (43 \times objective lens and 5 \times ocular), using an eyepiece equipped with a calibrated micrometer. The substrate is satisfactory if 90% of the particles do not exceed 2 μm in diameter and none exceeds 10 μm in diameter.

Buffer solution—Dissolve 60 mg of tris(hydroxymethyl)-aminomethane and 234 mg of sodium chloride in water to make 100 mL.

Bile salts solution—Prepare a solution to contain 80.0 mg of USP Bile Salts RS in each mL.

Standard test dilution—Suspend about 200 mg of USP Pancreatin Lipase RS, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the declared potency on the label of the USP Reference Standard. Maintain the suspension at 4° , and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Assay test dilution—Suspend about 200 mg of Pancreatin, accurately weighed, in about 3 mL of cold water in a mortar, triturate for 10 minutes, and add cold water to a volume necessary to produce a concentration of 8 to 16 USP Units of lipase activity per mL, based upon the estimated potency of the test material. Maintain the suspension at 4° , and mix before using. For each determination withdraw 5 to 10 mL of the cold suspension, and allow the temperature to rise to 20° before pipeting the exact volume.

Procedure—Mix 10.0 mL of *Olive oil substrate*, 8.0 mL of *Buffer solution*, 2.0 mL of *Bile salts solution*, and 9.0 mL of water in a jacketed glass vessel of about 50-mL capacity, the outer chamber of which is connected to a thermostatically controlled water bath. Cover the mixture, and stir continuously with a mechanical stirring device. With the mixture maintained at a temperature of $37 \pm 0.1^\circ$, add 0.1 N sodium hydroxide VS, from a microburet inserted through an opening in the cover, and adjust to a pH of 9.20 potentiometrically using a calomel-glass electrode system. Add 1.0 mL of the *Assay test dilution*, and then continue adding the 0.1 N sodium hydroxide VS for 5 minutes to maintain the pH at 9.0. Determine the volume of 0.1 N sodium hydroxide VS added after each minute.

In the same manner, titrate 1.0 mL of *Standard test dilution*.

Calculation of potency—Plot the volume of 0.1 N sodium hydroxide VS titrated against time. Using only the points which fall on the straight-line segment of the curve, calculate the mean acidity released per minute by the test specimen and the Standard. Taking into consideration the dilution factors, calculate the lipase activity, in USP Units, of the Pancreatin taken by comparison to the activity of the Standard, using the lipase activity stated on the label of USP Pancreatin Lipase RS.

Assay for protease activity (Casein digestive power)—

Casein substrate—Place 1.25 g of finely powdered casein in a 100-mL conical flask containing 5 mL of water, shake to form a suspension, add 10 mL of 0.1 N sodium hydroxide, shake for 1 minute, add 50 mL of water, and shake for about 1 hour to dissolve the casein. The resulting solution should have a pH of about 8. If necessary, adjust the pH to about 8, using 1 N sodium hydroxide or 1 N hydrochloric acid. Transfer the solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Use this substrate on the day it is prepared.

Buffer solution—Dissolve 6.8 g of monobasic potassium phosphate and 1.8 g of sodium hydroxide in 950 mL of water in a 1000-mL volumetric flask, adjust to a pH of 7.5 ± 0.2 , using 0.2 N sodium hydroxide, dilute with water to volume, and mix. Store this solution in a refrigerator.

Trichloroacetic acid solution—Dissolve 50 g of trichloroacetic acid in 1000 mL of water. Store this solution at room temperature.

Filter paper—Determine the suitability of the filter paper by filtering a 5-mL portion of *Trichloroacetic acid solution* through the paper and measuring the absorbance of the filtrate at 280 nm, using an unfiltered portion of the same *Trichloroacetic acid*

solution as the blank: the absorbance is not more than 0.04. If the absorbance is more than 0.04, the filter paper may be washed repeatedly with *Trichloroacetic acid solution* until the absorbance of the filtrate, determined as above, is not more than 0.04.

Standard test dilution—Add about 100 mg of USP Pancreatin Amylase and Protease RS, accurately weighed, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for about 25 minutes. Dilute quantitatively with *Buffer solution* to obtain a concentration of about 2.5 USP Units of protease activity per mL, based on the potency declared on the label of the Reference Standard.

Assay test dilution—Weigh accurately about 100 mg of Pancreatin into a mortar. Add about 3 mL of *Buffer solution*, and triturate for 5 to 10 minutes. Transfer the mixture with the aid of *Buffer solution* to a 100-mL volumetric flask, dilute with *Buffer solution* to volume, and mix. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to that of the *Standard test dilution*.

Procedure—Label test tubes in duplicate S_1 , S_2 , and S_3 for the standard series, and U for the sample. Pipet into tubes S_1 2.0 mL, into S_2 and U 1.5 mL, and into S_3 1.0 mL of *Buffer solution*. Pipet into tubes S_1 1.0 mL, into S_2 1.5 mL, and into S_3 2.0 mL of the *Standard test dilution*. Pipet into tubes U 1.5 mL of the *Assay test dilution*. Pipet into one tube each of S_1 , S_2 , S_3 , and U 5.0 mL of *Trichloroacetic acid solution*, and mix. Designate these tubes as S_{1B} , S_{2B} , S_{3B} , and U_B , respectively. Prepare a blank by mixing 3 mL of *Buffer solution* and 5 mL of *Trichloroacetic acid solution* in a separate test tube marked B . Place all the tubes in a 40° water bath, insert a glass stirring rod into each tube, and allow for temperature equilibration. At zero time, add to each tube, at timed intervals, 2.0 mL of the *Casein substrate*, preheated to the bath temperature, and mix. Sixty minutes, accurately timed, after the addition of the *Casein substrate* stop the reaction in tubes S_1 , S_2 , S_3 , and U by adding 5.0 mL of *Trichloroacetic acid solution* at the corresponding time intervals, stir, and remove all the tubes from the bath. Allow to stand for 10 minutes at room temperature for complete protein precipitation, and filter. The filtrates must be free from haze. Determine the absorbances of the filtrates, in 1-cm cells, at 280 nm, with a suitable spectrophotometer, using the filtrate from the blank (tube B) to set the instrument.

Calculation of potency—Correct the absorbance values for the filtrates from tubes S_1 , S_2 , and S_3 by subtracting the absorbance values for the filtrates from tubes S_{1B} , S_{2B} , and S_{3B} , respectively, and plot the corrected absorbance values against the corresponding volumes of the *Standard test dilution* used. From the curve, using the corrected absorbance value ($U - U_B$) for the Pancreatin taken, and taking into consideration the dilution factors, calculate the protease activity, in USP Units, of the Pancreatin taken by comparison with that of the Standard, using the protease activity stated on the label of USP Pancreatin Amylase and Protease RS.

Pancreatin Tablets

» Pancreatin Tablets contain not less than 90.0 percent of the labeled amount of pancreatin.

Packaging and storage—Preserve in tight containers, preferably at a temperature not exceeding 30°.

Labeling—Label the Tablets to indicate minimum pancreatin fat digestive power; i.e., single strength, double strength, triple strength.

USP Reference standards (11)—

USP Bile Salts RS

USP Pancreatin Amylase and Protease RS

USP Pancreatin Lipase RS

Microbial enumeration tests (61) and Tests for specified microorganisms (62)—Tablets meet the requirements of the test for absence of *Salmonella* species and *Escherichia coli*.

Disintegration (701): 60 minutes.

Assay for amylase activity (Starch digestive power)—Weigh and finely powder not fewer than 20 Tablets, avoiding the production of heat during the process. Proceed as directed in the *Assay for amylase activity* under *Pancreatin*, using as the assay preparation an accurately weighed portion of the powder, equivalent to 40 mg of pancreatin. (Use an inversely proportionate amount of the powder if the Tablets are labeled to contain a whole-number multiple of the minimum requirement for pancreatin digestive activity.)

Assay for lipase activity (Fat digestive power)—

Acacia solution, *Olive oil substrate*, *Buffer solution*, *Bile salts solution*, and *Standard test dilution*—Prepare as directed in the *Assay for lipase activity* under *Pancreatin*.

Assay test dilution—Proceed as directed for *Assay test dilution* in the *Assay for lipase activity* under *Pancreatin*, using as the assay preparation an accurately weighed portion of the powder, prepared as directed in the *Assay for amylase activity*, equivalent to about 200 mg of pancreatin.

Procedure and Calculation of potency—Proceed as directed in the *Assay for lipase activity* under *Pancreatin*.

Assay for protease activity (Casein digestive power)—

Casein substrate, *Buffer solution*, *Trichloroacetic acid solution*, *Filter paper* and *Standard test dilution*—Prepare as directed in the *Assay for protease activity* under *Pancreatin*.

Assay test dilution—Add an accurately weighed portion of the powder, prepared as directed in the *Assay for amylase activity*, equivalent to about 100 mg of pancreatin, to 100.0 mL of *Buffer solution*, and mix by shaking intermittently at room temperature for 25 minutes. Dilute quantitatively with *Buffer solution* to obtain a dilution that corresponds in activity to the *Standard test dilution*.

Procedure and Calculation of potency—Proceed as directed in the *Assay for protease activity* under *Pancreatin*.

Pancrelipase

» Pancrelipase is a substance containing enzymes, principally lipase, with amylase and protease, obtained from the pancreas of the hog, *Sus scrofa* Linné var. *domesticus* Gray (Fam. Suidae). It contains, in each mg, not less than 24 USP Units of lipase activity, not less than 100 USP Units of amylase activity, and not less than 100 USP Units of protease activity.

NOTE—One USP Unit of amylase activity is contained in the amount of pancrelipase that decomposes starch at an initial rate such that 0.16 μ Eq of glycosidic linkage is hydrolyzed per minute under the conditions of the *Assay for amylase activity*.

One USP Unit of lipase activity is contained in the amount of pancrelipase that liberates 1.0 μ Eq of acid per minute at pH 9.0 and 37° under the conditions of the *Assay for lipase activity*.

One USP Unit of protease activity is contained in the amount of pancrelipase that under the conditions of the *Assay for protease activity*, hydrolyzes casein at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid that gives the