

§ 429.12 Distinguishing colors on packages.

(a) The outside containers or wrappers of the packages, and the labels on the immediate containers of each potency of insulin injection shall be distinguished by the following colors:

Red, if it contains 40 U.S.P. Units of insulin per milliliter.

White, if it contains 100 U.S.P. Units of insulin per milliliter.

Narrow (at least 5 but not more than 20 to each inch) brown and white diagonal stripes, if it contains 500 U.S.P. Units of insulin per milliliter.

But if the master lot used was in crystalline form, the distinguishing colors, instead of those prescribed above, may be the following:

Red and gray, if it contains 40 U.S.P. Units of insulin per milliliter.

(b) The outside containers or wrappers of the packages, and the labels on the immediate containers of each potency of protamine zinc insulin suspension shall be distinguished by the following colors:

Red and white, if it contains 40 U.S.P. Units of insulin per milliliter.

Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

(c) The outside containers or wrappers of the packages, and the labels of the immediate containers of each potency of globin zinc insulin injection shall be distinguished by the following colors:

Red and brown, if it contains 40 U.S.P. Units of insulin per milliliter.

Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

(d) The outside containers or wrappers of the packages, and the labels of the immediate containers of each potency of isophane insulin suspension shall be distinguished by the following colors:

Red and blue, if it contains 40 U.S.P. Units of insulin per milliliter.

Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

(e) The outside containers or wrappers of the packages, and the labels of the immediate containers, of insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension shall bear a mark or design

to distinguish each drug, and each potency of these drugs shall be distinguished by the following colors:

Red and lavender, if it contains 40 U.S.P. Units of insulin per milliliter.

Black and white, if it contains 100 U.S.P. Units of insulin per milliliter.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974; 44 FR 55170, Sept. 25, 1979]

Subpart C—Product Standards**§ 429.25 Standards of quality and purity for protamine.**

When protamine is dried to constant weight at 100° C., its total nitrogen content is not less than 22.5 percent and not more than 25.5 percent, and its sulfate content, calculated as SO₄, is not less than 16 percent and not more than 19 percent.

§ 429.26 Standards of quality and purity for globin hydrochloride.

The ash content of globin hydrochloride is not more than 0.3 percent; its nitrogen content, calculated to moisture, ash, and hydrochloric acid free basis, is not less than 16.0 percent and not more than 17.5 percent.

Subpart D—Tests and Methods**§ 429.30 Tests and methods of assay.**

The following tests and methods of assay are prescribed for the purposes of the regulations in this part 429. (All reagents specified in this section shall be of U.S.P. quality or better.)

(a) *Tests and methods of assay for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension.* The tests and methods of assay for insulin injection, protamine zinc insulin suspension, globin zinc insulin injection, isophane insulin suspension, insulin zinc suspension, prompt insulin zinc suspension, and extended insulin zinc suspension shall be those set forth therefor in the U.S.P. or N.F., except that alternative test procedures may be employed when such have been authorized by the Commissioner.

(b) [Reserved]

(c) *Isophane ratio.* The isophane ratio shall be expressed as milligrams of protamine per 100 U.S.P. Units of insulin.

(1) *Reagents*—(i) *The stock buffer solution.* Dissolve in water the quantities of metacresol, phenol, glycerin, and disodium phosphate required to make 10 liters of the batch of isophane insulin and dilute to 1,000 milliliters.

(ii) *The insulin solution.* From a sample of the zinc-insulin crystals to be used in making the batch weigh a quantity which contains 10,000 U.S.P. Units of insulin. Dissolve the crystals in 15 milliliters of 0.1 percent hydrochloric acid. The resulting solution must be clear. Add it to 25 milliliters of the stock buffer solution (paragraph (c)(1)(i) of this section). Dilute with water to approximately 200 milliliters. Adjust the pH to 7.2 using hydrochloric acid or sodium hydroxide. The solution must be clear at this stage. If sodium chloride is to be used in preparing the batch add 25 milliliters of 4.2 percent (w/v) sodium chloride solution. Dilute to 250 milliliters with water. The pH must be between 7.1 and 7.4.

(iii) *The protamine solution.* Weigh 500 milligrams of the protamine to be used in making the batch and dissolve in 10 milliliters of the stock buffer solution (paragraph (c)(1)(i) of this section). If sodium chloride is to be used in preparing the batch add 10 milliliters of 4.2 percent (w/v) sodium chloride solution. Dilute with water to approximately 80 milliliters. Adjust the pH to 7.2 using hydrochloric acid or sodium hydroxide. Dilute with water to 100 milliliters. The pH must be between 7.2 and 7.4 and the solution must be clear.

(2) *Conduct of the test.* Measure six 25-milliliter samples of the insulin solution (paragraph (c)(1)(ii) of this section) into six tubes. To the first tube add 0.60 milliliter of the protamine solution (paragraph (c)(1)(iii) of this section), to the second add 0.72 milliliter, to the third add 0.84 milliliter, to the fourth add 0.96 milliliter, to the fifth add 1.08 milliliters, and to the sixth add 1.20 milliliters. Mix the contents of each tube and let stand for at least 30 minutes. Centrifuge. (Do not filter.) From each supernatant fluid remove two 10-milliliter samples, thus creating two series of samples. To each of one

series add 1 milliliter of the insulin solution (paragraph (c)(1)(ii) of this section). To each of the other series add 1 milliliter of the protamine solution (paragraph (c)(1)(iii) of this section). Mix each sample and let stand 10 minutes. Measure the turbidity of each sample by means of a photometer or nephelometer. Plot the readings of the two series of samples, using the amount of protamine originally added in milligrams per 100 U.S.P. Units of insulin as abscissas, and the photometer or nephelometer readings as ordinates. The abscissa of the intersection of the two curves indicates the isophane ratio of the protamine to the zinc-insulin crystals. In order to increase the precision of the test, when the approximate isophane ratio is known, the quantities of protamine solution to be added to the six tubes may be so chosen that the range (0.60 to 1.20 milliliters) is reduced, and the approximate isophane ratio is near the middle of the range.

The isophane ratio found is not more than 100 percent nor less than 90 percent of the ratio of protamine to insulin used in the trial mixture referred to in § 429.40(d)(7).

(d)–(e) [Reserved]

(f) *Chloride in globin hydrochloride*—(1) *Conduct of the test.* Weigh accurately approximately 0.5 gram of globin hydrochloride into a small beaker and dissolve in 10–15 milliliters of distilled water. Add 10 milliliters of tenth-normal silver nitrate, 5 milliliters of nitric acid, and 5 milliliters of a saturated solution of potassium permanganate. Stir and place on a steam bath for approximately 1 hour. If any brown color remains, stir again, rinse the sides of the beaker with distilled water and place on the steam bath until the brown color disappears. Transfer quantitatively to a 50-milliliter volumetric flask and fill the flask to the mark with distilled water. Mix and filter through a dry filter paper into a dry vessel. Transfer exactly 40 milliliters of the filtrate to a flask, add 2 milliliters of ferric ammonium sulfate test solution and titrate with tenth-normal ammonium thiocyanate. To obtain the percent chloride as HCl, subtract 1.25 times the number of milliliters of ammonium thiocyanate used from 10;

multiply this difference by 0.365 and divide by the weight of the sample in grams.

(2) *Reagents.* The reagents used are those described in the U.S.P.

(g) *Sulfate in protamine*—(1) *Conduct of the test.* Weigh accurately about 250 milligrams of protamine and dissolve it in about 100 milliliters of approximately tenth-normal hydrochloric acid. Heat to boiling and add 5 milliliters of barium chloride test solution. Digest on a steam bath for 1 hour; allow to cool. Filter through an ignited and weighed Gooch crucible; wash free of chlorides. Dry, ignite, and weight. The weight of barium sulfate thus obtained multiplied by 41.15 and divided by the weight of sample is the percent sulfate (SO₄) in the sample. Calculate the results to a moisture-free basis.

(2) *Reagents.* The reagents used are those described in the U.S.P.

(h) *Nitrogen.* Determine total nitrogen by the method described in the U.S.P., for insulin U.S.P.

(i) *Zinc in insulin-containing solutions or suspensions.* Use the method described in the U.S.P. for insulin injection.

(j) *Zinc in insulin-containing solids.* Dissolve 10 to 20 milligrams, accurately weighed, of insulin-containing solids in 5 to 10 milliliters of distilled water containing one drop of 5*N* hydrochloric acid, and proceed as directed in the U.S.P. under the test for zinc in insulin injection.

[39 FR 11750, Mar. 29, 1974, as amended at 39 FR 40286, Nov. 15, 1974]

Subpart E—Certification

§ 429.40 Requests for certification; samples; storage; approvals preliminary to certification.

(a) A request for certification of a batch is to be addressed to the Food and Drug Administration, Division of Research and Testing (HFD-470), 200 C St. SW., Washington, DC 20204.

(b) The initial request for certification submitted by any person shall be preceded or accompanied by a full statement of the facilities and controls used to maintain the identity, strength, quality, and purity of each batch, including a description of:

(1) The equipment, methods, and processes used in diluting master lots and parts thereof, and in maintaining the identity, strength, quality, and purity of master lots and dilutions therefrom;

(2) The tests and assays made on master lots and mixtures thereof, on dilutions and batches therefrom, and on ingredients used in such dilutions and batches; and

(3) The laboratory facilities used in such controls.

Such initial request shall also be preceded or accompanied by the keys to the master lot marks and batch marks used by such person. When any change is made in any of such facilities or controls, or in any such key, the next request for certification thereafter shall be accompanied by a full statement of such change.

(c) A person who requests certification of a batch shall submit in connection with his request statements showing:

(1) The master lot mark of each master lot used or to be used wholly or partly as an ingredient or component of an ingredient of the batch;

(2) The quantity of each such master lot so used;

(3) The original quantity of each such master lot (unless such information has been previously submitted);

(4) The quantity of the batch; and

(5) The batch mark.

(d) Except as otherwise provided in paragraphs (g) and (h) of this section, a person who requests certification of a batch shall submit in connection with his request and in the quantities hereinafter indicated, accurately representative samples of the following:

(1) The single master lot or the mixture of two or more master lots or parts thereof, to be used as ingredients of the batch; in a quantity containing approximately 10,000 U.S.P. Units of insulin, except that, if the batch is to be isophane insulin suspension, the quantity shall contain not less than 20,000 U.S.P. Units of insulin.

(2) If the batch is to be insulin injection, a trial dilution made from such master lot or mixture, glycerin, phenol or cresol, and hydrochloric acid, which dilution conforms to the standard of identity, strength, quality, and purity