Heparin Sodium, page 2204

Paragraph 2

Change

from: Heparin sodium intended for use in the manufacture of parenteral preparation contains not less than 180 IU per mg and heparin sodium not intended for the use in the parenteral preparation contains not less than 120 IU per mg, calculated on the dried basis.

to: Heparin sodium intended for use in the manufacture of parenteral preparation contains not less than 180 IU per mg for Heparin obtained from the intestinal mucosa or other suitable tissues of domestic mammals used for food by man except bovine source. Heparin sodium intended for use in the manufacture of parenteral preparation contains not less than 150 IU per mg obtained from the intestinal mucosa or other suitable tissues of bovine and heparin sodium not intended for the use in the parenteral preparation contains not less than 120 IU per mg, calculated on the dried basis.

Tests

Assay. *Potency*.

Insert following before Paragraph 1:
For Heparin sodium obtained from the porcine or other source except Bovine Source, perform the assay using method A. For Heparin Sodium obtained from bovine source, perform the assay using method A or B.
Method A.

Insert following at the end:

Method B.
Assay. Determine the potency of heparin sodium by comparing the concentration necessary to prevent the clotting of sheep or goat or human plasma with the concentration of the reference solution of heparin sodium necessary to give the same effect under the condition of the following method of assay.

Test solution. Dissolve 25 mg of substance under examination, in sufficient saline to produce a concentration of 1mg per ml, and dilute to a concentration estimated to correspond to that of the reference solution.

Reference solution. Determine by preliminary trial, if necessary, approximately the minimum quantity of heparin sodium RS which, when added in 0.8 ml of saline, maintains fluidity in 1ml of prepared plasma for 1 hour after the addition of 0.2 ml of calcium chloride (1 in 100). This quantity is usually between 1 and 3 Heparin Units. On the day of the assay prepare a reference solution such that it contains, in

each 0.8 ml of saline, the above-determined quantity of the reference standard.

Preparation of plasma. Collect blood from sheep directly into a vessel containing about 8 per cent of sodium citrate in the proportion of one volume to each 19 volumes of blood to be collected. Mix immediately by gentle agitation and inversion of the vessel. Promptly Centrifuge the blood, and pool the separated plasma. To a 1 ml portion of the pooled plasma in a clean test tube add 0.2 ml of calcium chloride (1 in 100), and mix. Consider the plasma suitable for use if a solid clot forms within 5 minutes. To store plasma for future use, subdivide the pooled lot into portions not exceeding 100 ml in volume, and store in the frozen state, preventing even partial thawing prior to use. For use in the assay, thaw the frozen plasma in a water-bath at a temperature not exceeding 37°. Remove particulate matter by straining the thawed plasma through a coarse filter.

Procedure. To meticulously clean 13 mm X 100 mm test tubes add graded amounts of the reference solution selecting the amount so that the largest does not exceed 0.8 ml and so that they correspond roughly to a geometric series in which each step is approximately 5 per cent greater than the next lower. To each tube so prepared add sufficient saline to make the total volume 0.8 ml. Add 1.0 ml of prepared plasma to each tube. Then add 0.2 ml of calcium chloride (1 in 100), note the time, immediately insert a suitable stopper in each tube, and mix the contents by inverting three times in such a way that the entire inner surface of the tube is wet.

In the same manner set up a series using the test solution, completing the entire process of preparing and mixing the tubes of both reference solution and the test solution within 20 minutes after the addition of the prepared plasma. One hour, accurately timed, after the addition of the calcium chloride, determine the extent of clotting in each tube, recognizing three grades (0.25, 0.50, and 0.75) between zero and full clotting (1.0). If the series does not contain 2 tubes graded more than 0.5, and 2 tubes graded less than 0.5, repeat the assay, using appropriately modified reference solution and test solution.

Convert to logarithms the volumes of reference solution used in the successive 5 or 6 tubes that bracket a grade of clotting of 0.5, including at least 2 tubes with larger and 2 tubes with a smaller grade than 0.5. Number and list the tubes serially, and tabulate for each the grade of clotting observed in each tube. From the log-volumes, x, and separately from their corresponding grades of clotting, y, compute the paired averages x_i and y_i of Tubes 1, 2, and 3, of tubes 2, 3, and 4, of Tubes 3, 4, and 5, and, where the series consists of 6 tubes , of Tubes 4, 5, and 6, respectively. If for one of these paired averages the average grade, y_i, is

exactly 0.50, the corresponding xi is the median log –volume of the reference solution x_s . Otherwise, interpolate xs from the paired values of y_i, x_i, and y_{i+1}, x_{i+1} that fall immediately below and above grade 0.5 as

$$x_s = x_i + (y_i - 0.5)(x_{i+1} - x_i)/(y_i - y_{i+1})$$

From the paired data on the tubes of the test solution, compute similarly its median log-volume x_U.

The log potency of the test solution is

$$M = x_s - x_u + \log R$$

Where $R = v_s/v_u$ is the ratio of the heparin Units (v_s) per ml of the reference solution to the mg (v_U) of heparin sodium per ml of the test solution.