Inactivation of Tetracycline with Sodium Borohydride

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Recently we reported on the rapid inactivation of tetracycline by use of cupric-morpholine complex (Kaplan, Lannon, and Buckwalter, J. Pharm. Sci., 54, 163, 1965). This degradation procedure is used as part of an assay technique for kanamycin in the presence of tetracycline. We have since found that sodium borohydride (Metal Hydrides Inc., Beverly, Mass.) can substitute for cupric-morpholine complex in this selective inactivation process. This paper reports on this new assay procedure.

In our laboratories, kanamycin is normally assayed for in the presence of tetracycline by use of a strain of Staphylococcus aureus which is resistant to tetracycline. Now, by use of sodium borohydride, both tetracycline and kanamycin may be assayed for in the presence of each other with a single organism, S. aureus 209P.

For example, a mixture containing an arbitrary equivalent of activity equal to 100 to 200 mg of tetracycline hydrochloride and 100 to 200 mg of kanamycin base is dissolved in 100 ml of 0.1 N hydrochloric acid. This is diluted to 200 to 500 ml with pH 4.5 phosphate buffer, and a sample is assayed for tetracycline content by the method of Grove and Randall (Assay Methods of Antibiotics, p. 48. Medical Encyclopedia, Inc., New York, 1955). Under these conditions, kanamycin does not interfere with the tetracycline assay.

For the kanamycin assay, a similar mixture of the above 0.1 N hydrochloric acid-tetracyclinc-kanamycin solutions is added to 100 to 500 ml of water, and the pH is adjusted to 8.0 to 9.5 with 10% sodium hydroxide; 200 to 400 mg of sodium borohydride dissolved in 25 ml of water are then added, and the solution volume is made up to 500 ml with water. This solution is held at ambient room temperature (22 to 25 C) for 1 hr to inactivate the tetracycline, and is then assayed for kanamycin by the method of Lamoy and Lannon (Antibiot. Ann. 1958-1959, p. 790, 1959). The large excess of sodium borohydride used does not affect the assay.

With the exception of cupric-morpholine complex, no other agent has been reported to inactivate tetracycline as rapidly and completely and at such mild conditions as does sodium borohydride.

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