Rapid Chemical Method for the Determination of Streptomycin in Feed Supplements

STANLEY E. KATZ

Department of Agricultural Chemistry, New Jersey Agricultural Experimental Station, New Brunswick, N. J.

A rapid chemical method has been developed for the determination of streptomycin in premix materials. The method is based upon the acid extraction of streptomycin and the conversion of the streptose moiety to maltol by base. The intensity of the absorbance is proportional to the concentration of the streptomycin present. Prepared feed supplements showed average recoveries of 98.3 to 99.2% of theoretical and coefficients of variation of 4.7 to 5.7%. Commercial premixes showed an average recovery of 103.5 to 109.4% with coefficients of variation of 3.2 to 6.4%. The method makes feasible the rapid and accurate determination of streptomycin in feed supplements.

At present, there are two methods available to the analyst for the determination of streptomycin in feeds: a microbiological procedure (*2*) and a chemical method (*1*). Although both methods can be used successfully for streptomycin in mixed feeds, neither method offers the increase in speed of analysis and/or greater accuracy that should be expected with the high levels of drug present in the concentrate. A method of analysis which is far more rapid yet reasonably accurate would simplify the problems of analyzing streptomycin-containing feed supplements.

A method is presented for determination of streptomycin which is both accurate and rapid. The proposed method is a simplification of a recently published chemical method (*1*) and is based upon the alkaline hydrolysis of the streptomycin molecule and conversion of the streptose moiety to maltol (*3*). The intensity of the absorbance of maltol is proportional to the concentration of streptomycin present.

Procedure

Preparation of a Calibration Curve. Prepare a calibration curve by transferring 1.0 to 6.0 ml. of a standard streptomycin solution containing 100 μg. of streptomycin per ml. into 10-ml. volumetric flasks. Add 1 ml. of 1*N* sodium hydroxide and make up to volume with distilled water. This will yield standards from 100 to 600 μg. Place the 10-ml. volumetric flasks in a 90° C. hot water bath for 5 minutes. Cool the flasks by placing them in an ice water bath. Determine the absorbance of each standard with a Beckman DU spectrophotometer at 324 mμ in 1.0-cm. silica cells with water as reference solution. Plot a standard curve from the observed absorbance values.

Determination of Streptomycin in Feed Supplements. Weigh a 1-gram sample of feed supplement into a 500-ml. Erlenmeyer flask fitted with a ground glass stopper. Add 100 ml. of 2% sulfuric acid (by volume) and shake mechanically for 30 minutes. Filter the contents through Whatman No. 42 filter paper. Dilute an aliquot of the filtered solution to such a volume that a 1- to 9-ml. aliquot will contain 100 to 600 μg. of streptomycin. Pipet two 1- to 9-ml. portions of the diluted solution into two 10-ml. volumetric flasks. Add 1 ml. of 1*N* sodium hydroxide to one of the 10-ml. volumetric flasks. Bring both the volumetric flasks to volume (if necessary) with distilled water. Place the flask whose contents were made basic (Flask I) in a 90° C. hot water bath for 5 minutes. Cool the heated flask, I, in an ice water bath. Centrifuge the contents of flask I, if necessary, prior to determining the absorbance of both flasks. Use water as the reference solution.

Subtract the absorbance of flask II from that of flask I and determine the quantity of streptomycin from a standard curve. Determine the total amount of streptomycin present from the ratio of the total volume of extracting liquid to the aliquot taken and from the ratio of the total volume of streptomycin containing dilution to the aliquot taken for analysis. The total micrograms found are equivalent to the parts per million. Conversion to grams per pound or any other method of expressing concentration can be made using the appropriate factor.

Results

Four premix materials containing fairly high levels of streptomycin were analyzed by the proposed method and the results examined for accuracy and reproducibility. Two of the samples were prepared for an A.O.A.C. (Association of Official Agricultural Chemists) collaborative study of a microbiological procedure for streptomycin, whereas the other two were samples of commercially available concentrates. It should be noted that per cent recoveries of the commercial premixes are based upon the tag guarantee, whereas the A.O.A.C. prepared supplement recoveries are derived from known quantities.

Table I. Recoveries of Streptomycin from Prepared Feed Supplements

Replication	Supplement A,[a] P.P.M.	Supplement B,[b] P.P.M.
1	6,000	29,400
2	6,796	27,540
3	6,120	28,080
4	6,336	27,800
5	6,336	24,580
6	6,228	26,340
7	5,936	26,200
8	5,828	25,800
9	6,496	29,400
10	6,176	26,460
Av.	6,125	27,160
Mean dev.	223	1,284
Stand. dev.	288	1,541
Coeff. of variation	4.7	5.7
Av. % recovery	99.2	98.3

[a] A.O.A.C. feed supplement containing 6,176 p.p.m.
[b] A.O.A.C. feed supplment containing 27,638 p.p.m.

Table I shows recoveries of the two A.O.A.C. prepared sample concentrates. Results obtained with Sample A showed an average recovery of 99.2% with a coefficient of variation of 4.7%. Sample B yielded results having an average re-

Table II. Recoveries of Streptomycin from Commercial Feed Supplements

Replication	Supplement C,[a] P.P.M.	Supplement D,[b] Mg./G.
1	41,025	563.8
2	43,025	492.8
3	43,225	606.4
4	40.650	528.8
5	42,025	506.6
6	45,575	500.9
7	42,275	512.2
8	43,125	512.9
9	43,750	504.5
10	42,925	521.3
Av.	42,760	525.0
Mean dev.	1,013	24.8
Stand. dev.	1,378	33.5
Coeff. of variation	3.2	6.4
Av. % recovery	103.5	109.4

[a] Tag guarantee: 18.75 grams per pound of streptomycin (equivalent to 41,300 p.p.m.) and 6.25 grams per pound of procaine penicillin.
[b] Tag guarantee: Each gram contains the equivalent of 480 mg. of streptomycin base.

covery of 98.3% and a coefficient of variation of 5.7%.

Table II presents results obtained from the two commercially available feed supplements. Supplement C yielded results having a 103.5% average recovery with a coefficient of variation of 3.2%. Supplement D showed results having a 109.4% recovery and a coefficient of variation of 6.4%.

The recoveries of all samples were essentially quantitative and possessed a good degree of reproducibility. It is important to note that a 1-μg. error in determining the aliquot concentration can become equivalent in the final calculation to hundreds of micrograms. The exact magnitude of such an error will be determined by aliquot size and concentration of premix.

Any materials possibly interfering are eliminated by means of the blank. The concentration of streptomycin is directly proportional to the difference in absorbance obtained from a heated portion of alkaline aliquot and unheated acid aliquot. The difference in absorbance readings is attributed to the conversion of the streptose moiety of the streptomycin molecule to maltol.

The analyst has a fair amount of latitude in the use of this method. Modification as to the volume of extracting acid and the aliquot size is possible for the solution of specific problems with little or no loss in accuracy and reproducibility.

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MEAT TENDERNESS FACTORS

Determination of Collagen in Raw and Cooked Beef from Two Muscles by Alkali-Insoluble, Autoclave-Soluble Nitrogen and by Hydroxyproline Content

SANFORD J. RITCHEY and SYLVIA COVER

Texas Agricultural Experiment Station, College Station, Tex.

Residual collagen, important in tenderness of meat, was measured in steaks of *L. dorsi* and *B. femoris* by micro-Kjeldahl nitrogen and by hydroxyproline following exhaustive extraction with alkali and conversion to gelatin by autoclaving. Collagen nitrogen values measured by hydroxyproline were consistently lower than those measured by micro-Kjeldahl in raw steaks, as well as in rare (61° C.) and well done (100° C.) steaks. Losses of collagen nitrogen were about the same in cooked steaks of the two muscles by each method of determination, but the two methods of assessment did not correspond. Losses were much greater in well done steaks than in rare steaks. *B. femoris* contained considerably more hydroxyproline and collagen than *L. dorsi*.

One of the factors believed to be involved in the tenderness or toughness of a piece of meat is the amount of residual connective tissue with collagen representing the major portion of this moiety. Studies on collagen from different species by Wohlisch (*16*), Gustavson (*6*), and Keech (*8*) demonstrated the effect of heat on this protein. When meat is cooked or heated, a fraction of the collagen is converted into gelatin; the amount not converted is believed to be one important factor in the over-all toughness of meat. Previous studies by Cover and Smith (*4*), Griswold and Leffler (*5*), and Irvin and Cover (*7*) indicate that the amount of residual connective tissue or collagen is dependent upon the method of cooking and the particular muscle or cut of meat. Irvin and Cover (*7*) found that *B. femoris* contained considerably more collagen than *L. dorsi*, and that the loss of collagen ranged from 20 to 25% when steaks from either muscle were cooked to an internal temperature of 61° C.

Numerous methods have been devised for the assessment of collagen. The method of Lowry, Gilligan, and Katersky (*9*) or some modification of this procedure is employed most frequently. This method depends upon the insolubility of collagen in dilute alkali and its conversion to gelatin on autoclaving. Collagen was found by weight in the original procedure, but Wilson, Bray, and Phillips (*15*) determined nitrogen in the autoclave-soluble fraction as a measure of collagen. Irvin and Cover (*7*) modified the method by using a more exhaustive extraction with water and alkali and then determining collagen by nitrogen in the autoclave-soluble fraction. This modification has given satisfactory data as far as consistency of duplicate samples is concerned, for cooked samples, as well as raw meat. When meat is heated, muscle proteins become more insoluble