IV. METHOD FOR DETERMINATION OF AMPROLIUM IN EGGS AND MEAT AND MEAT PRODUCTS OF CHICKENS

A. Principle

The sample is extracted by homogenization with dilute aqueous trichloroacetic acid and the filtrate is analyzed by a modification of the well-known thiochrome method for thiamine. Interference from thiamine is completely overcome by addition of silver nitrate before the ferricyanide oxidation step.

B. Reagents

1. Trichloroacetic Acid 5% - 5.0 grams reagent grade trichloroacetic acid per 100 ml of aqueous solution.

2. Sodium Hydroxide 30% - 30 grams of reagent grade sodium hydroxide per 100 ml of aqueous solution at room temperature.

3. Silver Nitrate 2% - 2.0 grams of reagent grade silver nitrate per 100 ml of aqueous solution.

4. Potassium Ferricyanide 2% - 2.0 grams of reagent grade potassium ferricyanide per 100 ml of aqueous solution. Store in a refrigerator.

5. Hydrogen Peroxide "3%" - 3.0 ml of reagent grade hydrogen 30% per 100 ml of aqueous solution. Prepare fresh daily.

6. n-Amyl Alcohol - Reagent grade.

7. Ethanol, Absolute - U.S.P.

C. Amprolium Standard

1. Stock Solution

Weigh accurately 20 mg of Amprolium Reference Standard and dilute with water in a volumetric flask to exactly 1000 ml and mix. Dilute 10.0 ml of this solution with water in a volumetric flask to exactly 1000 ml and mix. Each 5.00 ml is equivalent to 1.00 µg of Amprolium.

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C. Amprolium Standard (cont.)

2. Working Solutions

Transfer the appropriate volume of Amprolium Standard Solution (5.00, 10.00, and 20.00 ml) to a 50 ml volumetric flask. Add 25 ml of 10% trichloroacetic acid, dilute with water to 50.0 ml and mix. Each 5.00 ml is equivalent to 0.100, 0.200, or 0.400 µg of Amprolium.

D. Procedure

1. Sample

In the analysis of individual birds, use the entire liver (30-50 grams), the entire kidney (5-15 grams), or 50 grams of muscle tissue or skin plus associated fat.

2. Sample Preparation

Weigh the sample and add a measured volume of 5% trichloroacetic acid solution equivalent to 2 ml per gram of sample but not less than 15 ml.

Homogenize in a Waring Blendor or tissue homogenizer. Transfer to a 50 ml centrifuge tube and centrifuge at high speed. Filter the supernatent liquid through a small Pyrex wool plug in a funnel stem to remove any coarse suspended matter or oil.

3. Determination

Transfer an aliquot of 5.00 ml of the filtrate to 15 ml centrifuge tube. Add 2.00 ml of 30% sodium hydroxid solution, mix, add 0.20 ml of 2% silver nitrate solution, shake, and let stand for exactly two minutes. Add 1.00 ml of 2% potassium ferricyanide solution, mix, and let stand for exactly one minute. Add 0.20 ml of "3%" hydrogen peroxide solution, shake, and let stand for three minutes. Add 2.00 ml of n-amyl alcohol, shake 30 seconds, and centrifuge at high speed until the amyl alcohol layer is clear.

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D. Procedure

3. Determination (cont.)

Transfer 1.00 ml of the n-amyl layer to a small tube, add 0.20 ml of absolute ethanol and mix. Determine the fluorescence intensity with a spectrophotofluorometer using an activation wavelength of 400 mμ and a fluorescence wavelength of 460 mμ (or the proper Amprolium fluorescence maximum).

Run a reagent blank using 5.00 ml of 5% trichloroacetic acid solution in place of the filtered sample extract. Subtract this reading from the sample reading.

4. Standard Curve

Run Amprolium standards at suitable levels as follows: To 15 ml centrifuge tubes add 5.00 ml of the Amprolium Standard Working Solutions, respectively equivalent to 0.1, 0.2, and 0.4 μg of Amprolium, and continue under Determination beginning "add 2.00 ml of 30% sodium hydroxide solution....," etc.

5. Calculation

Calculate the Amprolium content of the tissue samples from observed readings and the standard curve.

6. Sensitivity

This method has a sensitivity of the order of 0.05 ppm.

All samples were read in a Perkin-Elmer Spectrofluorometer Model 204-S using micro-cells and a micro-cell turret obtained from Perkin-Elmer. Excitation and emission slit widths were 10 nm. Excitation wavelength was 400 mμ and optimum emission wavelength was determined to be 457 mμ. Table VII contains a summary of the data obtained from fortified turkey control samples used to verify the method. Table VIII contains the data for kidney assays; Table IX contains the data for liver assays; Table X contains the data for skin and fat assays; Table XI contains the data for muscle assays.