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Spectrophotometric Method for the Quantitative Analysis of Carbarsone in Turkey Feed

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Carbarsone is an antiambeic, antihistomonal drug effective in controlling and preventing blackhead disease in turkeys. It is generally provided as an additive to the birds' diet and is commercially available in feed products provided by the turkey growers' association. The drug has an LD_{50} of 510 mg/kg in mice, and in order to maintain an effective therapeutic level in the birds' feed the FDA has established a tolerance level of 0.00025 g of carbarsone/g of feed.

Because carbarsone has a potential for toxicity to the birds, it is important to have available an analytical method of detection which is both accurate and rapid. In the 1983 Feed Additive Compendium published by the Miller Publishing Company in cooperation with the Animal Health Institute (1983) there is a qualitative procedure for carbarsone which has been found to be both time-consuming and inefficient. In addition, as it is qualitative only, the range of detection for the drug is limited. Whitmoyer Laboratories, Inc. manufactures carbarsone and has used a silver diethyldithiocarbamate method for analyses (1978). This method is also involved and time-consuming. The purpose of this paper is to present a less time-consuming, more sensitive and more accurate analysis for carbarsone.

EXPERIMENTAL

Carbarsone has an arsanilic group in its chemical structure. Attempts to use the official first action method for arsanilic acid found in the Association of Official Analytical Chemists methods book was only partially successful (1975). The following describes an outline of modifications made to the arsanilic acid procedure which resulted in a rapid and dependable analysis for carbarsone.

Reagents

a) Acetate buffer solution - dissolve 5 g of anhydrous NaOAc in 50 ml of distilled H_2O. Add 6

ml glacial HOAc and bring up to 100 ml with distilled H_2O.

b) 1.0N NaOH - 10 g NaOH/250 ml distilled H_2O.
c) Concentrated HCl.
d) 0.1% $NaNO_2$/25 ml distilled H_2O.
e) 0.5% ammonium sulfamate - 0.125 g ammonium sulfamate/25 ml distilled H_2O.
f) 0.1% NED (N-1-ethlene diamine dihydrochloride)-0.025 g NED/25 ml distilled H_2O.
g) Carbarsone standard - Whitmoyer Laboratories, 19 North Railroad Street, Myerstown, PA 17067.

ANALYTICAL PROCEDURE

If the amount of carbarsone is known then the sample weight should be calculated to contain approximately 0.002 g of carbarsone. However, even for residue analysis no more than 8 g should be used due to the limitations of the glassware volumes. The weighed sample is put into a 200 ml volumetric flask and 80 ml of distilled H_2O added, followed by 2 ml of 1.0N NaOH. The flask is placed in a shallow pan of boiling water for 10 min, swirling every 3 min. Slowly add 20 ml of concentrated HCl to the flask which is then swirled and cooled to room temperature. The sample is then brought to volume with distilled H_2O and filtered through a Whatman filter #40. Five ml of clear filtrate is pipetted into each of two 50-ml glass centrifuge tubes. To each tube is added 2 ml of 0.1% $NaNO_2$, the solutions are mixed and allowed to set for 5 min. To each tube is then added 2 ml of 0.5% ammonium sulfamate. The solutions are swirled and allowed to set for 2 min. To one tube only is added 1 ml of 0.1% NED. To the other tube is added 1 ml of distilled H_2O. Both solutions are swirled and allowed to set for 10 min. Reading should be done immediately after 10 min since the color reaction tends to fade.

Instrument and operating conditions. A Baush and Lomb Spectrophotometer 20 was used at 540 nm. The reference was distilled H_2O, and all readings were read on the absorption scale.

Standards preparation. The dry carbarsone was kept under refrigeration and the standard solutions were prepared daily. The stock solution was made up in acetate buffer solution. The working solutions were made up with distilled H_2O. The concentration of

the standard matched the concentration of the carbarsone of the sample (if known). The standard was run along with the sample starting with two 5 ml volumes pipetted into each of two 50-ml glass centrifuge tubes.

RESULTS AND DISCUSSION

Three analyses were done in duplicate for carbarsone extracting it from different feed media during one year. The results are shown in table I.

TABLE I

Recoveries of different levels of carbarsone

Feed media	Expected amount (g carbarsone/g feed)	Results (avg of two analyses)
Turkey feed with carbarsone	0.00025 g/g	0.00028 g/g
Turkey feed without carbarsone (but suspect contamination)	?	0.000058 g/g
Non-medicated feed (fortified)	0.00025 g/g	0.00025 g/g

Although the analysis for carbarsone follows the analytical procedure for arsanilic acid the concentration of the final color reaction is considerably less for carbarsone. Therefore, the dilution factors for carbarsone are different from those for arsanilic acid analysis. Furthermore, carbarsone is more rapidly dissolved in an acetate buffer solution than in the NaOH solution used to dissolve arsanilic acid standards. In addition there does not seem to be an observable difference in using distilled H_2O as a reference blank instead of an acetate buffer solution reference blank. The minimum detectability so far observed is 58 ppm.

The objective of this analysis developed around

a known concentration of carbarsone in a partially defined media. However, it would be of future interest to analyze environmental substrates collected from surrounding areas of a turkey industry to determine if concentrations of carbarsone are being released to the environment.

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