Colorimetric Determination of Cadmium Anthranilate in Feedstuffs

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If cadmium anthranilate, an anthelmintic, is added to a swine ration it must be kept within a narrow range of concentrations. Its presence must be readily and accurately measured. A procedure has been developed for the determination of microgram amounts of cadmium anthranilate. The method is designed for use with feedstuffs containing this compound. A coupling reagent is added to the feed extract to form a pink compound having an absorption maximum at 538 mμ. Special conditions for extraction, removal of interferences, and development of color are described.

CADMIUM ANTHRANILATE, as a feed additive, is intended solely for use as an anthelmintic for swine. It must be alone in a prepared feed, at a 0.044% level. The material has been registered by the Texas Feed Control Service, thus rendering necessary a method of analysis which is both sensitive and rapid. Several methods in the literature may be utilized for the determination of cadmium anthranilate, but these depend on the analysis of the cadmium portion. There is no published method designed especially for the determination of cadmium anthranilate.

An electrolytic deposition method for cadmium, described by Scott (*5*) and adapted for feeds and concentrates by Stone (*7*), entails complete digestion of the feed with nitric, sulfuric, and perchloric acids. The solution is then treated in the usual manner for electroplating onto a platinum gauze electrode. A second method by Stone (*8*) utilizes the same preparation technique, but the cadmium is determined with dithizone. A previous report of this method was made by Klein and Wichmann (*1*) who investigated cadmium as a contaminant in foods. The dithizone method for cadmium is described in detail by Saltzman (*4*), who also gives instruction for removing the effect of interfering ions. Perhaps the most precise methods for determining cadmium is the polarographic technique described by Kolthoff and Lingane (*2*). A direct ashing procedure may be utilized, followed by a dithizone extraction to remove interfering cations.

The following method is a direct colorimetric determination of cadmium anthranilate which proceeds without destruction of the compound or the feed sample. It is based on the color reaction of the primary amine of the compound instead of the metal moiety.

Experimental

Cadmium anthranilate presents two primary amino groups as functional centers for sites of diazotization. Initial attempts to form a color complex with a diazotized product failed to produce colors with sufficient intensity for analytical use. The color reaction proceeded slowly and reached a maximum color only after several hours. To hasten this equilibrium, samples were placed in a hot water bath. Several temperatures and heating periods were tried. The most satisfactory was found to be 30 minutes at 65° C.

Some difficulty was experienced in the extraction of cadmium anthranilate from feeds. The product is not appreciably soluble in any solvent unless slightly acidified. The use of acidified acetone, alcohol, or other water-soluble organic solvent invariably led to cloudy solutions at the color formation step. It was found that the compound could be quantitatively extracted from feeds using a 1% aqueous hydrochloric acid solution. The extract was filtered with suction, through a mat of Super-Cel on a Büchner funnel.

Certain components of feeds may dissolve in the extraction step and need to be removed. These dissolved materials interfere with the production of a stable color. Charcoal was used first in an attempt to purify the extract. Interferences, and the cadmium anthranilate too, were removed by use of the charcoal. A column packed with alumina was next tried. The extract was passed through the column and the eluate tested for the presence of cadmium anthranilate. The result was positive; however, the first 10 ml. of eluate contained less cadmium anthranilate than the second 10 ml. This effect was studied and it was found that reproducible results could be obtained by using the first 50 ml. of eluate.

Improved sensitivity was obtained by diluting the aqueous solutions to 50% with alcohol. Acetone gave the same results, but often resulted in a cloudy solution.

Method

Standard Solutions. Weigh 0.2000 gram of cadmium anthranilate into a 100-ml. volumetric flask. Add 5 ml. of concentrated hydrochloric acid and 20 ml. of distilled water to effect solution. Dilute to the mark with distilled water. Dilute 10.0 ml. of this solution to 100 ml. in a volumetric flask. The second standard solution contains 200 γ per ml.

Standard Curves. Transfer 0-, 2-, 4-, 6-, 8-, and 10-ml. portions of the second standard solution to 100-ml. volumetric flasks and dilute to volume with 1% hydrochloric acid solution. Transfer a 5.0-ml. aliquot from each flask to a test tube. Add 5.0 ml. of ethyl alcohol, 1.0 ml. of a 1 to 1 hydrochloric acid solution, and 1.0 ml. of a 0.1% sodium nitrite solution to each test tube and mix well. The sodium nitrite solution should be prepared fresh daily. After 5 minutes add 1.0 ml. of a 0.5% solution of ammonium sulfamate to each tube and mix. Make fresh ammonium sulfamate at least once a month. Finally after 2 minutes add 1.0 ml. of the coupling reagent [0.1% aqueous solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride] to each tube and mix. The coupling reagent is stable for one week when kept in a brown bottle. The final volume is 14.0 ml.

Place the tubes in a 65° C. water bath for 30 minutes. Cool the tubes in a large beaker of tap water. Then read the samples at 538 mμ in a spectrophotometer. Plot absorbance *vs.* concentration. The resulting standard curve covers the range from 0 to 100 γ which are present in the final 5.0-ml. aliquot, and represents the theoretical curve under ideal conditions.

To prepare a standard curve that will be representative of conditions that are present when analyzing feed samples, a blank feed from several brands of chicken and turkey feeds, both mashes and pelleted, is prepared. These are mixed, ground together, and remixed. Care is taken to choose feeds with a wide variety of ingredients and to avoid feeds that contain other medicaments.

Weigh 2.0 grams of feed into each of six 100-ml. volumetric flasks. Pipet 0-, 2-, 4-, 6-, 8-, and 10-ml. portions of

the second standard solution onto the samples and make to volume with 1% hydrochloric acid solution. Shake intermittently over a 30-minute period. Allow the heavy particles to settle and filter the extract with suction through a 1/4-inch mat of Super-Cel held on a filter paper in a Büchner funnel. Use a 60-mm. internal diameter, Büchner funnel and a 250-ml. suction flask, and filter paper (S & S 597, 5.5 cm.). Discard the first few milliliters of filtrate used to wet the mat of Super-Cel. The filtrate must be clear. Treat as described under "Standard Curves" starting with "transfer a 5.0-ml. aliquot."

Analysis of Samples. Samples are handled as described for standard curves, without the addition of the cadmium anthranilate. A blank should be run with each set of samples, just as the blank with the standard curve was run. A large amount of the blank feed should be kept on hand and used as such for all medicated feed analysis. The blank feed serves to duplicate the conditions that are present in the feed containing the medicament.

Discussion. Analysis of feeds containing cadmium anthranilate should pose no problem as to interfering materials, because no other medicament has been approved for use in the same feed. The method described removes interferences that are normally present in feeds. The blank made from the laboratory prepared blank feed further reduces chances for error. The standard curve itself, being prepared in the presence of a blank feed, serves as proof of recovery of the material from feeds. Additional recovery data are presented in Table I. All data were collected using a Beckman DK-2 ratio recording spectrophotometer.

Table I. Recovery of Cadmium Anthranilate from Blank Feed

Cadmium Anthranilate in Final Aliquot		Recovered,
Added	Recovered[a]	%
10.0	10.0	100.0
20.0	20.0	100.0
30.0	29.0	96.6
40.0	39.0	97.5
50.0	47.5	95.0

[a] Average of 4 determinations.

An $E^{1\%}_{1\ cm.}$ value of 1737 from a theoretical standard curve compares well with an $E^{1\%}_{1\ cm.}$ value of 1710 as calculated from a standard curve made in the presence of a feed extract. An $E^{1\%}_{1\ cm.}$ value of 2427 is reported for arsanilic acid by Senn and Woolford (*6*). Merwin (*3*) reports that feeds containing large amounts of tryptophan tend to give high blanks when the method is used for determining arsanilic acid. The same possibility exists here, but to a large extent is obviated by the use of a feed blank as previously discussed.

Acknowledgment

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DRIED FRUITS STABILITY

Modified Direct Colorimetric Method for Determination of Sulfur Dioxide in Dried Fruits

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A simple and direct colorimetric method for the determination of sulfur dioxide in dried fruits can be conveniently used in the field, as well as in the laboratory. The procedure is rapid and reproducible, giving values comparable with those obtained by the gravimetric Monier-Williams technique.

PRECISE DETERMINATION of sulfur dioxide in dried cut fruits and golden, bleached raisins is important to the dried fruit industry. To enhance the stability of these products, it is necessary to maintain the sulfur dioxide content above certain minimum levels. Analytical procedures commonly used now are the gravimetric method of Monier-Williams (*1*) and the iodometric method of Nichols and Reed (*8*). These methods include a distillation operation, and the Monier-Williams method is rather involved and time-consuming for some industrial applications. A simpler procedure for sulfur dioxide analysis has been sought for some time.

Detailed reviews of the chemistry and analytical methods for sulfur dioxide in foods have been published by Joslyn and Braverman (*7*) and Gehman and Osman (*4*). A number of iodometric methods have been described (*2, 9, 10*) for sulfur dioxide determination in dried fruits and vegetables. Colorimetric methods for the determination of sulfur dioxide in some food products have also been reported by several authors (*3, 5, 6, 11*). These methods are based on Steigman's (*12*) technique for the determination of sulfuric acid by modification of the well-known reaction for the detection of aldehydes with the colorless solution of fuchsine–sulfurous acid.

Very recently West and Gaeke (*14*)

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