The test for Amprol cannot be run directly on the finished feed. It is necessary to extract the compound with 65% methanol and filter the extract.

In general, the dimethylformamide–sodium methylate test was most satisfactory as a field procedure for detection of Zoamix in either premixes or finished feed. The reagents are very stable and can be used for long periods of time. The sodium methylate test was therefore adapted to the determination of the distribution of Zoamix in the finished feed. In the preparation of Zoamix, the chemical is coated on small particles of soybean meal. When the meal is mixed with the other feed ingredients, these particles are distributed throughout the feed. When a small quantity of the feed is spread uniformly between two pieces of filter paper and is pressed on a pad moist with dimethylformamide and sodium methylate, the compound appears as green areas. The color is transferred to the paper when pressure is exerted on the system, and visual indication of the distribution of Zoamix is obtained (Figure 2). This test is simple to operate and the equipment—i.e., Petri dishes, test tubes, etc.—can be used over and over again by simply washing and drying it between each test. This procedure has been employed to study the distribution of Zoamix in various lots of commercially prepared feed and has been very helpful in determining when the sample is well mixed.

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FEED ADDITIVES

Colorimetric Determination of Procaine Penicillin in Premixes

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A colorimetric method has been developed for determining procaine penicillin in premix materials. It is based upon the conversion by base of penicillins to penicilloic acids. The penicilloic acids yield penaldic acid derivatives and penicillamine when treated with mercuric ions. Penicillamine is oxidized to the disulfide by phosphomolybdic acid which is converted into molybdenum blue. The intensity of the blue color is proportional to the concentration of penicillin present. Laboratory prepared feed supplements showed average recoveries of 93.9 to 104.5% of the theoretical with coefficients of variation ranging from 3.4 to 6.1%. Commercial premixes showed average recoveries of 98.8 to 110.3% of tag guarantees with coefficients of variation ranging from 2.8 to 4.3%.

When used in animal nutrition, procaine penicillin is ordinarily available in supplement form, consisting of a uniform mixture of procaine penicillin in a suitable carrier. The use of premixes permits greater accuracy and makes for greater ease in handling the small amounts of procaine penicillin required.

At present, procaine penicillin in premixes is determined by a microbiological cylinder-plate procedure (*4*). This method yields results that range from 84.0 to 115.2% of procaine penicillin added, based on 95% confidence limits and takes 18 to 24 hours for completion. The chemical method of analysis proposed herein offers a more rapid and accurate means of determining procaine penicillin in premix materials. This method is based upon the Pan procedure (*3*) of simultaneously determining penicillins and penicilloic acids. It is well established that penicillins yield penicilloic acids when treated with base, Equation 1 (*2*). When treated with mercuric ions, the penicilloic acids or alkali-inactivated penicillins form penaldic acid derivatives and penicillamine, Equation 2 (*1*). Since penicillamine is a mercaptan, it is oxidized to the disulfide by phosphomolybdic acid which gives molybdenum blue (*1*). The intensity of the molybdenum blue is proportional to the concentration of penicillin present.

Reagents

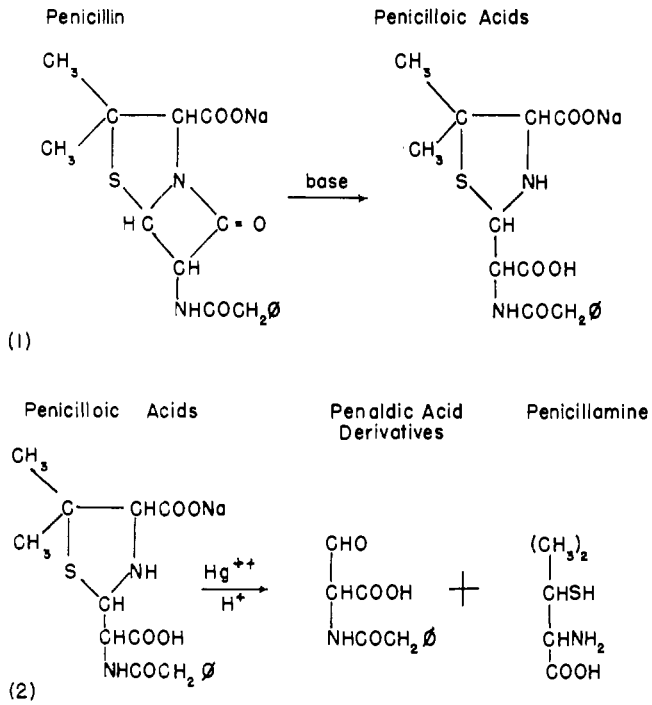
Nelson's Color Reagent A. Dissolve 25 grams of ammonium molybdate in 450 ml. of distilled water, add 21 ml. of concentrated sulfuric acid, and mix. Add 3 grams of disodium arsenate, $Na_2HAsO_4 . 7H_2O$, dissolved in 25 ml. of water. Mix and place in an incubator at 37° C. for 24 to 48 hours.

Mercuric Chloride Solution B. Dissolve 0.7 gram of reagent grade mercuric chloride per liter of distilled water.

Mixed Color Reagent. Mix 3.5 ml. of reagent A, 4.0 ml. of reagent B, and 2.5 ml. of water. Prepare daily.

Procedure

Establishment of Calibration Curve. Weigh sufficient procaine penicillin standard into a 100-ml. volumetric flask so that the resulting solution will contain 100 μg. of procaine penicillin per ml. (1 mg. of procaine penicillin is equivalent to 0.6 mg. of penicillin G, master standard). Pipet aliquots of the standard solution (in the range of 100 to 1000 μg.) into 25-ml. volumetric flasks. Add 1 ml. of *1N* sodium hydroxide to each 25-ml. volumetric flask, shake, and allow to stand for at least 15 minutes. Acidify with 1 ml. of *2N* sulfuric acid and mix well. Add 1 ml. of the mixed color reagent and mix well; bring to volume and allow to stand for 30 minutes. Measure the intensity of the resulting blue color at 740 mμ with a Beckman DU spectrophotometer or any other suitable spectrophotometer using a rea-



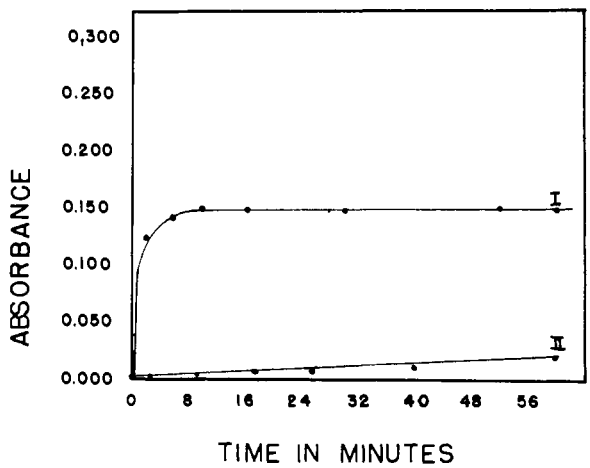


Figure 1. Reduction of arsenomolybdate by (I) penicillin (II) alkali-inactivated penicillin at the 20-μg. level

Table I. Recovery of Procaine Penicillin from Laboratory Prepared Premixes

Replication	Found, P.P.M.					
	Premix I	Premix II	Premix III	Premix IV	Premix V	Premix VI
1	6,680	8,472	42,460	10,442	6,977	11,883
2	6,780	8,986	42,060	10,242	7,026	10,640
3	6,038	8,590	38,105	9,898	7,026	10,635
4	6,038	8,166	39,290	10,145	6,580	10,692
5	6,532	8,908	38,595	10,640	6,433	10,964
6	6,285	8,828	39,090	10,292	6,977	10,902
7	6,630	8,116	38,895	10,392	7,225	12,991
8	6,335	7,838	38,105	10,935	6,779	10,711
9	6,482	7,522	36,915	10,540	7,274	10,901
10	6,185	8,908	38,300	9,648	6,829	11,186
Av.	6,398	8,428	39,181	10,317	6,912	11,150
Mean dev.	222	419	1,253	271	204	522
Std. dev.	264	509	1,578	352	265	678
Av. % recovery	96.4	93.9	104.5	95.1	93.9	96.9
Coeff. of variation	4.1	6.1	4.0	3.4	3.8	6.1

Premix I contains 3.1037 grams/pound or 6,638 p.p.m. of procaine penicillin.
Premix II contains 4.0667 grams/pound or 8,967 p.p.m. of procaine penicillin.
Premix III contains 17.7885 grams/pound or 37,479 p.p.m. of procaine penicillin.
Premix IV contains 4.9237 grams/pound or 10,845 p.p.m. of procaine penicillin.
Premix V contains 3.3432 grams/pound or 7,364 p.p.m. of procaine penicillin.
Premix VI contains 5.2125 grams/pound or 11,500 p.p.m. of procaine penicillin.

gent blank as a reference solution. Prepare a standard curve.

Determination of Procaine Penicillin in Premixes. Weigh 1 gram of the premix into a 125-ml. Erlenmeyer flask fitted with a ground-glass stopper. Add 25 ml. of 95% methanol and extract by shaking mechanically for 30 minutes. Filter the extract through Whatman No. 40 filter paper. Pipet an aliquot containing 200 to 800 μg. of procaine penicillin into a test tube. Aliquot generally will be 1 or 2 ml. Add 1 ml. of 1*N* sodium hydroxide; shake, and allow to stand for at least 15 minutes. Add 1 ml. of 2*N* sulfuric acid and mix well. Filter through Whatman No. 42 filter paper into a 25-ml. volumetric flask. Wash the test tube several times with 2-ml. portions of distilled water and pass the washings through the filter paper. Wash the filter paper with distilled water. Add 1 ml. of the mixed color reagent to the filtrate, mix well, and make up to volume with distilled water. Allow to stand for 30 minutes and measure the color at 740 mμ with a spectrophotometer. Compare with the standard curve and determine the micrograms of procaine penicillin in the aliquot. From the aliquot size, the total volume of extracting solvent, and the weight of sample taken, calculate the parts per million of procaine penicillin. Conversion to grams per pound can be made by use of the appropriate factor.

Results

The laboratory premixes were prepared by blending carefully weighed amounts of procaine penicillin standard in a twin-shell blender with wheat middlings or solvent-extracted soybean meal as a diluent. The samples were ground prior to analysis. Table I shows the results of 10 replications of six laboratory prepared premixes. Recoveries ranged from 93.9 to 104.5% of the theoretical amounts of procaine penicillin added with coefficients of variation ranging from 3.4 to 6.1%.

Commercially available premixes were collected during the feed inspection program of the New Jersey Agricultural Experiment Station. It must be noted that the per cent recoveries of procaine penicillin are based upon the tag guarantee and not upon recoveries of known quantities. The results obtained from the two commercial samples examined showed recoveries of 98.8 and 110.3% of tag guarantee with coefficients of variation of 2.8 and 4.3%, respectively (Table II). The precision and accuracy of the commercial samples are very close to the results obtained from the laboratory premixes.

This method measures penicillins as well as some of the breakdown products. To measure the extent of the breakdown products penicilloic acid or penicillamine, the procedure can be altered slightly, as follows. Equal aliquots of the extract are placed in two test tubes. One tube is labeled "sample" and follows the usual procedure of base hydrolysis, acidification, filtration, color development, and measurement of color intensity. The other tube is labeled as the "blank" and the contents diluted to 10 ml. with distilled water and filtered through Whatman No. 42 filter paper into a

Table II. Recovery of Procaine Penicillin from Commercially Available Premixes

Replication	Found, P.P.M.	
	Premix A	Premix B
1	11,415	15,540
2	11,081	15,340
3	11,430	15,885
4	11,016	14,400
5	10,545	14,498
6	10,920	15,430
7	10,856	14,598
8	10,502	15,440
9	11,119	14,995
10	10,897	14,845
Av.	10,878	15,187
Mean dev.	235	520
Std. dev.	310	652
Av. % recovery	98.8	110.3
Coeff. of variation	2.8	4.3

Premix A tag guarantee: 5 grams/pound or 11,013 p.p.m. of procaine penicillin and 15 grams/pound of streptomycin.

Premix B tag guarantee: 6.25 grams/pound or 13,765 p.p.m. of procaine penicillin and 18.75 grams/pound of streptomycin.

25-ml. volumetric flask. One milliliter of the mixed color reagent is added and the solution made up to volume with distilled water. After a 10-minute color development period, determine the intensity of the color. Centrifuge prior to determining the color intensity, if necessary. The difference between the absorbance value for the sample and that of the blank can be attributed to penicillin.

The 10-minute color development period in the modification was chosen because the reduction of the arsenomolybdate reagent by penicillin is minimal, while the reduction of the arsenomolybdate by the oxidation of penicillamine to the disulfide is maximal (Figure 1). Any penicilloic acid extracted from the premix will be cleaved to produce penicillamine and penaldic acid. Any penicillamine extracted as well as produced will be oxidized rapidly to the disulfide prior to significant reduction by penicillin alone.

In the premixes studied, the absorbance values in the modification ranged from 0.015 to 0.022 with the exception of premix II, which had a high value of 0.059. This abnormally high value is probably attributable to the high concentration of penicillin, 37,479 p.p.m.

In comparing the procedure and the modification, extremely small differences were found for the penicillin levels in the laboratory prepared premixes or the commercial premixes. Because of the minute differences, the determination of breakdown products was ignored in the determinations.

The analyst has considerable latitude in choosing sample size and volume of extraction solvent, but an aliquot of greater volume than 2 ml. is not recommended.

The advantages of this method are: simplicity, accuracy, a multiplicity of samples can be analyzed, and procaine penicillin can be determined accurately without the use of microbiological procedures.

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FEED ADDITIVES

Stability of Several Oleandomycin Derivatives in Livestock and Poultry Feed Products

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Since its discovery in 1954, oleandomycin has proved to be a highly effective chemotherapeutic agent. It also elicits substantial growth responses in livestock and poultry. However, the common pharmaceutical derivatives were unstable in feed products, which made their agricultural application unfeasible. Extensive laboratory screening and plant-scale evaluation resulted in the development of a new oleandomycin resin adsorbate for agricultural use. This product exhibits feed stability which equals or surpasses that of many other commercial antibiotics, while retaining full biological activity.

OLEANDOMYCIN (Matromycin, Chas. Pfizer & Co.), first announced by Sobin, English, and Celmer in 1954 (*18*), has received wide acceptance in clinical therapy. When this antibiotic was evaluated as a growth promoter for chickens and turkeys, excellent responses were obtained at levels as low as 2 grams per ton of feed (*2, 4, 13, 16, 17, 19–21*). Substantial growth increases over control birds were observed during 5 years of repeated tests under conditions where penicillin fed to parallel groups often elicited only slight responses. Oleandomycin also exerted strong growth-promoting action in swine, cattle, and sheep (*8, 9, 14, 15*). When it appeared that oleandomycin might find application in commercial feeds as a growth promoter, the product formulation laboratory at the Pfizer Agricultural Research Center attempted to develop a suitable feed supplement containing this antibiotic.

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Materials and Methods

Stability studies comprise laboratory-scale tests and commercial pelleting operations.

Laboratory-Scale Tests. Five-pound portions of a master basal ration were supplemented with each test antibiotic product and thoroughly mixed in a Patterson-Kelley twin-shell blender. About 0.5 pound was withdrawn for initial assays, and the remaining portion transferred to amber glass screw cap bottles for elevated temperature storage. Bottles were kept sealed during storage