A New Colorimetric Procedure for the Determination of Benomyl

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Two residual analytical methods for determining the fungicide benomyl in crops have been published by Pease and Gardiner (1) one colorimetric and the other based on spectrofluorometry. Both these methods as well as the one described here, actually consist in a combined determination of the fungicide and metabolites hydrolyzable to 2-aminobenzimidazole. The fluorometric method has lately been improved by Pease and Holt (2) who state that the colorimetric procedure is a difficult and time-consuming one, an opinion shared by other analytical chemists. The fluorometric procedure which may be excellent, involves use of expensive instrumentation for the present not common in residue laboratories. Pellizzari and Gaiter (3) discovered that addition of hypochlorite or hypobromite to an aqueous solution of 2-aminobenzimidazole caused the appearance of an intense blue colour. Work was started to evaluate their discovery as the basis for an analytical method. A suitable procedure and discussion of the method is given here.

Method

Instrument

Beckman DB spectrophotometer equipped with recorder and glass optical cells with 10mm light-path.

Reagents

Sodium hypochlorite solution (commercial solution containing about 12 per cent of available chlorine) mixed with four times its volume of water.

2-aminobenzimidazole, supplied by E.I. du Pont de Nemour Co. Inc., Wilmington, Del. Aqueous solution prepared by dissolving the substance in a small volume of alcohol and diluting with water.

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Procedure

Follow the extraction, hydrolysis and clean-up procedure of Pease and Gardiner including the last evaporation before the instrumental determination. This evaporation is carried out in a 50ml beaker. Stop the evaporation when so much of the ethyl acetate is evaporated that the liquid remaining is essentially an aqueous solution. If the air is so dry that no water condenses during the evaporation of ethyl acetate, add aproximately 1 ml of water. Roll the beaker gently to wet its sides. Pour the water into a 10 ml graduated centrifuge tube. Wash the beaker with 3 ml of hot water and add it to the tube. Allow to cool. Adjust the volume in the tube to 5 ml. Transfer part of the contents to an optical cell. Measure at 560 nm against water as referance. If the absorbance is higher than 0.015, the sample ought to be discarded. Add 2 drops of sodium hypochlorite solution using an eyedropper. Put the cell quickly into the compartment of the colorimeter without mixing. As the colour develops and fades rather rapidly, it is recommended to use a recorder. Compare the absorbance with a standard curve obtained by measuring aqueous solutions of 2-aminobenzimidazole to quantify. 2 µg/ml is a feasible lower starting point for the construction of the curve.

Results and discussion

In spite of the rapid fading of the colour, the absorbance versus concentration of 2-aminobenzimidazole shows good agreement with Beer's law up to 60 µg/ml. The change in colour from blue through green to brownish yellow qualitatively indicates that the substance is the amine searched for. Since the hypochlorite solution is added to the sample solution directly in the optical cell, background colour can be measured before addition of the colour developing reagent. The clean-up is so efficient that one can afford to discard samples when the absorbance exeeds 0.015. In difficult cases the additional sodium hydroxide wash of Pease and Holt is a valuable supplement to the clean-up. If the uncertainty span is confined to three times the absorbance mentioned above, the limit of detection is slightly above 2 µg/ml 2-aminobenzimidazole which corresponds to 0.5 ppm benomyl in a 50g sample based on a 5 ml final volume.

Under favourable circumstances this volume can be reduced but very seldom to 1 ml leading to 0.1 ppm, the sensitivity claimed by Pease and Gardiner for their methods. Benomyl-residue values published by Purokoski (4) are based on their colorimetric procedure, and he does, however, give limits of detection varying from 0.4 to 1.0 ppm.

In order to check precision of the method four different crops were fortified with 2 ppm of benomyl and analyzed.

Results are given in table 1.

TABLE 1.

Recovery and standard deviation for analysis of crops fortified with benomyl.

Crops	Recovery	Stand.Dev.
Apple	82%	10%
Cabbage	93%	8%
Cucumber	80%	12%
Onion	84%	9%

The method has also been applied to other kinds of fruits and vegetables; black currants, raspberries, strawberries, carrots, celery and leeks with acceptable recovery but the number of spiked samples of each kind were limited.

References

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