cohol to each flask to give a volume of 10 ml. Add 5 ml. of 5N hydrochloric acid to each flask and proceed with the hydrolysis and color development as described above.

Figure 2 shows a typical calibration curve.

Discussion

The zinc reduction step after the hydrolysis greatly reduces the amount of extraneous color present in some extracts. A 5-minute reflux is sufficient for most samples, but onions require a 15-minute reflux period.

The filtrate obtained after zinc reduction, when diluted with distilled water, develops a turbidity due to precipitation of lipide materials. The addition of Celite and filtration remove them completely.

The determination from which the coupling reagent is omitted allows for correction due to extraneous color in the solution.

The reproducibility of the method was tested by macerating a large number of tomatoes in isopropyl alcohol as previously described (Table II).

The puree was thoroughly mixed and divided into 12 equal portions by weight. Each portion contained the equivalent of 100 grams of tomatoes and 100 ml. of isopropyl alcohol. Two samples were analyzed without the addition of Dyrene and the other nine were divided into three groups. Each group was treated with various amounts of Dyrene.

In spite of the low absorbance values read, the average deviation in the concentration range of 0.5 to 2.5 p.p.m. is about 5%.

To test the efficiency of the method, Dyrene in isopropyl alcohol was added to plant tissue in the blending operation and carried through the entire procedure (Table III).

The values for untreated control material show considerable variation. In the samples analyzed, untreated controls gave values which were equivalent to 1.0 p.p.m. or less. The reproducibility of values from any particular location was good. In using the method for residue determination, the values for the untreated controls are always subtracted before reporting the results. An initial concentration of 1 p.p.m. gives an absorbance in the final solution of 0.02 using the dilutions and aliquots described in the method. Readings of untreated controls ranged up to values equivalent to 1 p.p.m. of Dyrene.

The recovery data (Tables II and III) justify using 1.0 p.p.m. as the limit of sensitivity of the method. In view of the low toxicity of Dyrene, this sensitivity is considered satisfactory.

For concentrations of Dyrene of less than 5 p.p.m., the final readings should be made in 10-cm. quartz cells. By reading in this way, the sensitivity of the method can be extended to 1 p.p.m.

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FUNGICIDE RESIDUES

Determination of Dyrene in Apples by Application of the Zincke Reaction

DAVID L. BARRY and DONALD J. LISK

Pesticide Residue Laboratory, Department of Entomology, New York State College of Agriculture, Cornell University, Ithaca, N. Y.

A method is presented for the determination of small amounts of the fungicide, Dyrene, in apples. Apple tissue is extracted with acetone and the acetone filtrate is extracted with chloroform. The evaporated extract is taken up in benzene and chromatographed on alumina to remove interferences. The Zincke reaction with pyridine and alkali is used to determine Dyrene. Recovery of Dyrene, added to apples, averaged 97 ± 10%. About 0.05 p.p.m. of Dyrene can be detected, if the entire chloroform extract from a 100-gram sample of apple is analyzed. Analysis of apples treated with Dyrene for scab control shows residues of about 0.1 p.p.m.

DYRENE, 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine, controls certain foliage diseases of higher plants. Its effectiveness against apple scab and rust prompted the development of a rapid analytical method for its determination in apple fruit.

Meagher *et al.* (*3*) have developed a colorimetric method for the determination of Dyrene residues in plant material. It is based on the acid hydrolysis of Dyrene to *o*-chloroaniline followed by diazotization and coupling with N-1-naphthylethylenediamine.

Burchfield and Schuldt (*1*) have shown that the colorimetric reaction of pesticides containing active halogen with pyridine followed by alkali (Zincke reaction) can be used for their quantitative determination. Burchfield and Storrs (*2*) used the Zincke reaction to determine 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine in solvent extracts of fungus spores. In the work reported Dyrene is extracted from apple tissue by the method of Meagher and MacDougall (*4*) for Guthion, using acetone and chloroform. The evaporated extract is taken up in benzene and the solution is passed through an alumina column to remove interfering substances. The color reaction, essentially that of Burchfield and Storrs (*2*), is then used to determine Dyrene.

Reagents

DYRENE STOCK SOLUTION. Dissolve 0.1000 gram of analytical standard Dyrene (Chemagro Corp., P. O. Box 4913, Kansas City, Mo.) in C.P. acetone. Transfer to a 500-ml. volumetric flask, make up to volume with acetone, and mix. One milliliter of this solution contains 200 γ of Dyrene.

DYRENE STANDARD SOLUTION. Dilute 5 ml. of the stock solution to 100 ml. with acetone in a volumetric flask. One milliliter of this solution contains 10 γ of Dyrene.

Procedure

Preparation of Standard Curve (0 to 100 γ of Dyrene). Pipet aliquots of the Dyrene standard solution corresponding to from 0 to 100 γ of Dyrene into a series of 20 × 150 mm. test tubes. Evaporate the solution to dryness on a

water bath, using a stream of air. Remove the test tubes from the water bath, add 2 ml. of purified pyridine, and allow the reaction to occur for 20 minutes. Add 10 ml. of redistilled petroleum ether and pour the mixture into a 60-ml. separatory funnel. Rinse the test tube into the separatory funnel with another 10-ml. portion of petroleum ether. Add exactly 10 ml. of distilled water and shake. Allow the layers to separate and collect the lower water layer. If the aqueous layer is cloudy, filter it through Whatman No. 40 filter paper. Take a 5-ml. aliquot of the water layer and transfer it to a 0.5-inch (outside diameter) cuvette for the Bausch & Lomb Spectronic 20 spectrophotometer. From this point, treat each sample separately through the color development. Add 3 ml. of 3N sodium hydroxide. Measure the per cent transmittance at 440 mμ, exactly 2 minutes after the addition of the alkali and water in the reference cell.

Preparation of Alumina. Pour 250 ml. of 0.1N hydrochloric acid into a 600-ml. beaker. Add 250 grams of alumina (General Chemical Co. aluminum oxide, ignited powder). Stir well and allow it to stand for 30 minutes. Decant the supernatant liquid and fine particles. Transfer the alumina to a tray lined with aluminum foil and heat overnight in an oven at 100° C. Store the alumina in a tightly stoppered bottle.

Analysis of Apples. Dice 100 grams of apple tissue and blend for 3 minutes in 100 ml. of C.P. acetone. Add another 100-ml. portion of acetone and blend for 3 minutes more. Transfer the mixture to a 1-quart Mason jar. Rinse the blender with two 50-ml. portions of acetone and add them to the Mason jar. Seal the jar and tumble the mixture for 30 minutes. Filter the acetone-apple mixture through glass wool in a 100-mm. powder funnel and collect the filtrate in a 1-liter separatory funnel. Rinse the Mason jar with 100 ml. of acetone and pour through the filter. Add 300 ml. of water to the separatory funnel followed by 100 ml. of C.P. chloroform. Shake the mixture gently for 30 seconds and allow the layers to separate. Drain off the lower chloroform layer and collect in a 600-ml. beaker. Shake the acetone solution six times more with 50-ml. portions of chloroform and combine all the chloroform extracts in the beaker. Emulsions can be troublesome here. Carry out the first two or three chloroform extractions, with gentle shaking. Break the emulsions which may form, by allowing them to stand or by agitating with a stirring rod. Break up persistent emulsions by centrifuging. Filter the chloroform solution through a 100-mm. powder funnel, filled with anhydrous sodium sulfate on a plug of glass wool, and collect the filtrate in a 1-liter graduated cylinder. Rinse the funnel with three 25-ml. portions of chloroform and combine the rinses with the chloroform filtrate. Mix the chloroform solution and record the volume. Take an appropriate aliquot (possibly 200 ml.) of the solution and transfer it to a 250-ml. Erlenmeyer flask. Evaporate to dryness in a water bath using a stream of air.

Preparation of Column. The column consists of a glass tube (13-mm. inside diameter), 18 inches long, and drawn out at one end to an inside diameter of about 5 mm. Seal a stopcock at this end. Insert a plug of glass wool into the tube until it rests just above the constricted portion. Fill the tube with C.P. benzene and add alumina until the height is 6 inches, after settling. Drain off the benzene until its level is just at the top of the alumina. Do not allow it to fall below, or air will enter the column.

Chromatography and Color Development. Dissolve the residue, after evaporation of the chloroform, into 5 ml. of benzene and pour the mixture on the alumina column. Rinse the flask with 5 ml. of benzene and add it to the column. Force the sample on the column with 1 to 2 pounds of air pressure. After the sample has entered the column, fill the tube with benzene and, using air pressure, collect the first 40-ml. fraction in a 25 $\times$ 200 mm. test tube. Evaporate the benzene to dryness using an air stream and a water bath. Proceed as in the preparation of the standard curve.

Results and Discussion

Acetone, chloroform, and benzene (chemically pure grade) were used throughout this study. The suitability of redistilled technical grade solvents was not investigated.

The extraction procedure is essentially that of Meagher and MacDougall (*4*). In the Guthion procedure (*4*) hydrochloric acid is used to eliminate chlorophyll interference. This is not necessary in this procedure.

The temperature of the water bath used for evaporation of the solvent was 80° C. Loss of Dyrene, under these conditions, was not appreciable.

The depth of the alumina on the tray, during the activation of the alumina, was about 0.5 inch. The cleanup using alumina was necessary. It eliminated interfering substances which led to high check values when the pyridine and alkali were added directly to the evaporated chloroform extract.

The standard curve follows Beer's law from 0 to 100 γ Dyrene. The absorbancy index of Dyrene is 2.22 absorbance units per mmole-mm., with a coefficient of variation of 7.3%. The absorbancy index was calculated using the molecular weight of Dyrene, because the exact composition of the reaction product was unknown.

Table I. Recovery of Dyrene from Apples

Dyrene Added, γ	
50	100
Recovery, %	
90	109
96	102
104	113
80	87
88	99
124	90
	85

The recovery of Dyrene added to apple tissue is shown in Table I. Dyrene was added to the apple-acetone mixture before blending. The average recovery of Dyrene was 97 $\pm$ 10%. Based on established tolerances for Dyrene in vegetables, the successful recovery of 0.5 p.p.m. of Dyrene from apples makes the method adequate for regulatory purposes.

During 1957, five applications of Dyrene were made on Cortland apple trees at the rate of 2 pounds per 100 gallons of water in a special formulation of 50% wettable powder. The first application was made on May 21 and the fifth on July 3. The ripe apples were harvested on September 15 when the Dyrene was determined. Analysis of duplicate samples showed residues of 0.06 and 0.14 p.p.m. of Dyrene. Analysis of untreated apples showed no residue.

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