The number of consecutive titrations possible with the same supporting electrolyte depends upon the rate at which the electrodes became contaminated.

Table III indicates the amount of variation obtained from consecutive titrations of standard potassium bromide solution using 0.5*N* perchloric acid in 80% ethanol as supporting electrolyte. With a ground solution of 0.5*N* nitric acid in 60% ethanol, the electrodes became contaminated more quickly, but this more convenient supporting electrolyte may be used if only a few titrations are to be carried out.

Different concentrations of standard potassium bromide were titrated to determine the standard deviation for the 1- and 5-ma. generating current ranges (Table IV). Concentrations of bromide below those given in Table IV may be determined by using the 1-ma. generating range, but the deviation increases until a sensitivity limit of about 3 μg. is reached. Recoveries of methyl bromide (Table V) range from 97 to 99.7%, depending upon the amount taken.

Figure 3 illustrates the change in concentration of methyl bromide as a function of time at various points in a fumigation chamber. The chambers were packed with jute bales and sampling ports were arranged so that methyl bromide–air mixtures could be drawn from the center of the bales and the free space around them.

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HERBICIDE RESIDUES

Separation and Colorimetric Determination of Monuron and Diuron Residues

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Diuron residues are determined by minor modifications of a colorimetric method for determining monuron residues. Monuron and diuron residues are determined simultaneously in the presence of each other by an extension of the liquid chromatographic technique. The method is sensitive to 2 μg. of either compound and will recover both compounds quantitatively in the presence of a wide variety of plant tissue and soil. If only one of the two compounds is present, the procedure will identify it.

Weed killers based on 3-*p*-chlorophenyl-1,1-dimethylurea (monuron and 3-(3,4-dichlorophenyl)-1,1-dimethylurea) (diuron) are useful on both agricultural and nonagricultural sites for control of a wide range of annual and perennial weed species. When applied to the soil at low rates, they selectively control germinating annual weeds in certain crops. They are used at higher rates where residual control of all vegetation is desired.

The two chemicals may be used alternatively for selective weed control in an increasing number of crops. Thus, a sensitive residue method is needed to determine which chemical is present or to determine each compound if both are present in the same plant tissue.

A method for the determination of microgram quantities of monuron in a wide variety of plant tissues (*2*) has been modified for the determination of diuron residues. It involves the quantitative hydrolysis of the urea herbicide, under reflux conditions in a strongly alkaline medium, to the corresponding aromatic amine with simultaneous partitioning of the amine into an organic solvent. The amine is then extracted into dilute acid and determined colorimetrically after diazotization and coupling reactions.

Many plant tissues contain small amounts of naturally occurring materials, such as tryptophan, which will also respond to this procedure. This interference can be eliminated by chromatographically separating the azo dye derived from *o*-aminoacetophenone (hydrolysis product of tryptophan) from that formed from *p*-chloroaniline (*1*) or 3,4-dichloroaniline.

The chromatographic technique has now been extended to separate the azo dye derived from *p*-chloroaniline from that derived from 3,4-dichloroaniline. This paper describes the latter technique and the modified colorimetric procedure which makes possible essentially simultaneous determination of monuron and diuron. Recovery data are presented for various plant tissues to which known quantities of both urea herbicides were added (Table I).

This method is complementary to one discussed by Kirkland (*3*.)

Reagents and Apparatus

p-Chloroaniline, recrystallized from 70% aqueous ethanol.

3,4-Dichloroaniline. recrystallized from 70% aqueous ethanol.

Sodium hydroxide solution, 20% aqueous, prepared from reagent grade chemical.

Hydrochloric acid, 1*N* solution.

Dow Corning Antifoam A, emulsion.

n-Hexane, washed twice with $^1/_{10}$ its volume of 2*N* hydrochloric acid.

Sodium nitrite, 2% aqueous solution, prepared fresh daily.

Sulfamic acid, 10% aqueous solution, prepared fresh daily.

N-(1-Naphthyl)ethylenediamine dihydrochloride, Fisher Scientific Co. (N-21), 2% aqueous solution, prepared fresh daily.

Hydrochloric–acetic acid mixture, equal volumes of 1*N* hydrochloric acid and glacial acetic acid.

Table I. Recovery of Monuron and Diuron from Various Crops

Plant Tissue	Weight of Sample, Grams	Monuron Added μg.	Monuron Added P.p.m.	Monuron Found μg.	Monuron Found P.p.m.	Monuron % Recovery	Diuron Added μg.	Diuron Added P.p.m.	Diuron Found μg.	Diuron Found P.p.m.	Diuron % Recovery
Oranges	500	55.4	0.11	52.5	0.11	95	123	0.25	114	0.23	93
		139	0.28	126	0.25	91	45.2	0.09	45.1	0.09	100
		102	0.20	95.5	0.19	94	280	0.56	254	0.51	91
Pineapple	200	12.1	0.06	12.8	0.06	106	18.7	0.09	18.3	0.09	98
		63.6	0.32	59.4	0.30	93	43.2	0.22	41.5	0.21	96
		84.8	0.42	78.0	0.39	92	108	0.54	103	0.52	95
Sugar cane	200	14.0	0.07	14.0	0.07	100	11.1	0.06	11.5	0.06	104
		42.4	0.21	41.0	0.21	97	64.8	0.32	59.0	0.30	91
		106	0.53	100	0.50	94	64.8	0.32	62.3	0.31	96
Grapes	300	20.2	0.07	18.7	0.06	93	11.1	0.04	10.8	0.04	97
		63.6	0.21	60.5	0.20	95	86.4	0.29	82.2	0.27	95
		212	0.71	199	0.66	94	108	0.36	99.0	0.33	92
Asparagus	100	20.2	0.20	19.5	0.20	97	29.8	0.30	30.6	0.31	103
		106	1.1	97.5	0.98	92	216	2.2	200	2.0	93
		318	3.2	296	3.0	93	216	2.2	210	2.1	97

Apparatus for continuous extraction of steam-volatile substances. Distillation-extraction head constructed as shown in Figure 1.

Flask, round-bottomed, Monel metal, 2-liter capacity, 29/42 TS.

Funnel, separatory, 1-liter capacity with short beveled tip.

Spectrophotometer, Beckman Model B or equivalent.

Chromatographic Column. Tightly pack Whatman cellulose powder No. 3199-U into a 24-inch length of 15-mm. O.D. glass tubing to a height of 20 inches. Use penicillin assay disk as a packing support at each end of the column.

Column Adapter. Join two one-hole rubber stoppers, sizes 0 and 7, back to back with a 3-inch section of 6-mm. O.D. glass tubing. Extend the tube about one half the distance into the size 0 stopper.

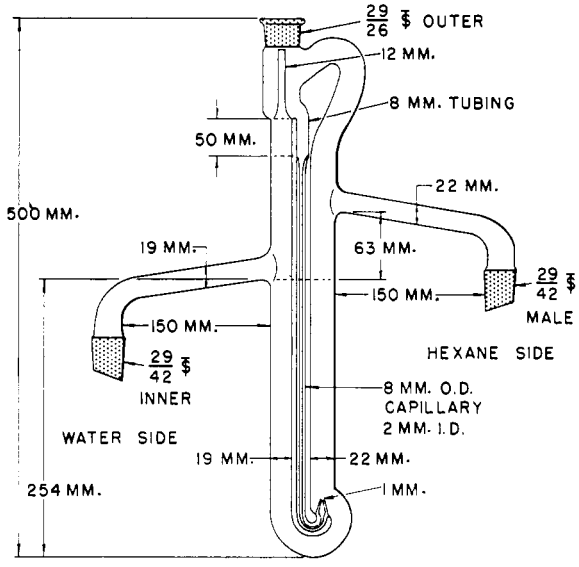

Figure 1. Combination steam distillation–extraction apparatus

Calibration

Prepare standard solutions containing 5 μg. per ml. of *p*-chloroaniline (PCA) and 3,4-dichloroaniline (DCA) in 1*N* hydrochloric acid and transfer a series of aliquots containing 5 to 80 μg. of each aniline to 50-ml. volumetric flasks. Dilute each to approximately 40 ml. with 1*N* acid and add 5 ml. of glacial acetic acid. Pipet 1 ml. of 2% sodium nitrite solution into each flask, mix, and allow to stand for 15 minutes. Destroy excess nitrite by adding 1 ml. of 10% sulfamic acid solution, mixing, and allowing to stand for 10 minutes. Add 2 ml. of 2% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution, dilute to volume with 1*N* acid, mix, and allow 15 minutes for full color development.

Determine the absorbance of each solution at 560 mμ, using 1-cm. cells and distilled water as the reference solution. Plot absorbance *vs.* micrograms of chlorinated aniline added to give calibration curves that are used directly for subsequent determinations.

Procedure

Place 100 to 500 grams of representative tissue in a 2-liter Monel metal flask, and add several boiling chips, 2 teaspoonfuls of Dow Corning Antifoam A, and 1000 ml. of 20% sodium hydroxide solution. Fill the U-tube of the distillation-extraction head to the level of the lower arm with distilled water and attach the flask to the lower arm. Add 500 ml. of acid-washed hexane and a boiling chip to the 1-liter round-bottomed flask and attach to the upper arm. With a 24-inch water-cooled condenser in position, apply heat to both flasks at such a rate that condensed hexane and water pass through the capillary in the form of small sausages. Allow digestion-extraction to proceed for about 12 hours (overnight is convenient).

At the completion of the digestion-extraction period, cool the hexane and transfer it quantitatively to a 1-liter separatory funnel having a short, beveled tip. Extract the aromatic amine with six 15-ml. portions of 1*N* hydrochloric acid, collecting the extracts in a 100-ml. volumetric flask. Dilute to volume with the 1*N* acid.

Transfer an aliquot containing from 2 to 80 μg. of aniline (maximum of 40 ml.) to a 50-ml. volumetric flask, add 5 ml. of glacial acetic acid, and develop color by diazotizing and coupling reactions as described under Calibration.

Transfer 25 ml. of the colored solution to a cellulose powder column attached to a 500-ml. vacuum flask by a column adapter. Apply suction to the system to obtain a reasonable flow rate, prior to the addition of the sample.

Develop the column by washing with four to five 250-ml. portions of 1*N* hydrochloric acid. The dye derived from *o*-aminoacetophenone separates almost immediately, moving ahead of the other dyes, and washes completely through the column. The PCA dye is adsorbed in the lower half of the column and the DCA dye forms a completely separate zone at the top half.

Drain the column, remove the suction, and cut the column appropriately to isolate the desired dyes. Insert new penicillin disks in the shortened sections, and elute the dye by passing 15 to 20 ml. of 1 to 1 (v./v.) mixture of 1*N* hydrochloric acid and glacial acetic acid through the column. Collect the colored solution in a 25-ml. volumetric flask and dilute to volume with the 1 to 1 mixture. Measure absorbance at 560 mμ in 1-cm. cells, using distilled water as the reference solution. Determine micrograms of chlorinated aniline from the appropriate calibration curve and calculate:

$$\text{P.p.m. of monuron} = \frac{\mu\text{g. of PCA in final aliquot} \times 1.56 \times \text{aliquot factor}}{\text{weight of sample in grams}} \quad (1)$$

$$\text{P.p.m. of diuron} = \frac{\mu\text{g. of DCA in final aliquot} \times 1.44 \times \text{aliquot factor}}{\text{weight of sample in grams}} \quad (2)$$

Discussion

Inclusion of a chromatographic cleanup with the direct caustic hydrolysis procedure resulted in a method for determining a few micrograms of monuron and/or diuron residues with quantitative recoveries from a variety of plant tissues. The method is applicable to a wide range of residue concentrations.

Blanks of untreated plant tissues are not a problem, as the azo dye derived from naturally occurring interfering materials is easily eluted from the chromatographic column, resulting in an essentially no-blank method. The dyes derived from *p*-chloroaniline and 3,4-dichloroaniline may also be separated using a longer column (24-inch to replace the 12-inch column) (*1*) and sufficient 1*N* hydrochloric acid to develop the column completely. The *p*-chloroaniline dye is adsorbed in the lower half of the column, and the 3,4-dichloroaniline dye forms a completely separate zone at the top half. Each band may be eluted quantitatively from the column, and the color intensity measured individually and converted to the respective urea herbicide content.

If the sample being analyzed shows only one colored band, the urea herbicide present is determined from the position of this dye on the column. The identity of the compound may be confirmed by forming the dye on a separate aliquot of the aniline solution to which *p*-chloroaniline has been added. The relative position of the dyes on the column will verify the urea herbicide present.

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FEED ADDITIVES ANALYSIS

Field Tests for the Identification of Coccidiostats in Premixes and Finished Feeds

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A series of simple colorimetric procedures has been developed for the identification of the commercial coccidiostats in premixes and finished feeds. These tests can be used in the field to determine which coccidiostat is present in unknown samples of feed. A procedure is described for adapting those tests to the determination of the distribution of Zoamix or other coccidiostats in finished feed which can be used to ascertain how complete the mixing operation has been.

In poultry management, it is often necessary to determine if a given coccidiostat is present in various lots of premix and finished feed, and to ascertain how well the compound is blended in the product. Most of the procedures which have been developed for the analysis of the various coccidiostats in feed product are time-consuming and are designed to be used with samples of more or less known composition. If the composition of the feed is not known, it often requires a considerable amount of time and effort to ascertain which coccidiostat is present using conventional, analytical procedures. In commercial operation, it is desirable to have a simple spot test which may be used in the field to identify the coccidiostat present in the feed.

During the past several years, it has been observed that Zoalene (3,5-dinitro-*o*-toluamide, the Dow Chemical Co. trademark, except in the United States and Canada) could be distinguished from other coccidiostats on the basis of several simple color reactions (*2*, *3*). These reactions have been adapted so that they can be used directly with premixes or finished feed to determine the presence of the compound. The procedures employed may easily be used in the field. It was decided to extend these procedures to determine if a series of color tests could be developed to distinguish between the major coccidiostats now on the market.

The present paper deals with some of the observations which have been made using a series of spot tests. In most cases, the tests employed are modifications of analytical procedures which have been previously developed for the routine analysis of a given coccidiostat. Attempts have been made to distinguish the various coccidiostats on the basis of positive reactions given by each compound.