Colorimetric Microdetermination of 1-Chloro-2-nitrobenzene in Pineapple

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The herbicide, 1-chloro-2-nitrobenzene, is extracted from pineapple tissue and determined colorimetrically by measuring the intensity of the azo dye formed after reduction, diazotization, and coupling with *N*-(1-naphthyl)-ethylenediamine. This sensitive micromethod can determine 0.01 p.p.m. of the herbicide in pineapple.

A METHOD for the determination of 1-chloro-2-nitrobenzene (Monsanto Chemical Co.'s herbicide *o*-nitrochlorobenzene, ONCB) in pineapple tissue in connection with residue studies is needed. The method described below is similar to that used by Averell and Norris (*1*) for the determination of parathion and by Young and Gortner (*3*) for the estimation of monuron after hydrolysis to *p*-chloroaniline. Sherwood (*2*) suggested the application of the parathion method to the analysis of ONCB.

Experimental

Reagents. Attapulgus clay–Celite adsorbent. Mix two parts of Attapulgus clay with 1 part of Celite.

Sodium nitrite, 1% solution (w./v.). Keep in refrigerator and discard after a week.

Ammonium sulfamate, 10% solution (w./v.). Keep in refrigerator and discard after a week.

N-(1-Naphthyl)-ethylenediamine dihydrochloride, 1% solution (w./v.). Filter before using and keep in refrigerator. Discard after a week.

o-Nitrochlorobenzene standard, melting point 32.3° C. (Monsanto Chemical Co. No. A61).

Procedure. Assemble opposite longitudinal wedges of at least four fruits from a replicate treatment to make a sample. Cut into small pieces, mix, and weigh 150 grams into a Waring Blendor. Add 100 ml. of water and 150 ml. of benzene, blend for 1 minute or until the sample is homogenized, and pour the mixture into two 250-ml. centrifuge bottles. Stopper tightly with cork stoppers, centrifuge at 1500 r.p.m. for 10 minutes, and siphon off 100 ml. of the supernatant liquid.

Remove pigments, which appear to be completely extracted by the benzene, by passing the extract through a column of Attapulgus clay–Celite adsorbent prepared as follows: Place a wad of glass wool at the bottom of a 125-ml. open-top cylindrical separatory funnel or a chromatographic column of similar size and add a mixture of 5 grams of adsorbent and about 20 ml. of benzene. Apply suction until the benzene is just removed from the top of the column. Do not suck dry, as this will allow the extract to channel subsequently.

Collect the decolorized extract in a 200-ml. tall-form beaker and wash the column with 60 ml. of benzene. Add an acid-washed Hengar boiling stone, cover, and evaporate the mixture by boiling on a hot plate at low heat until about 10 ml. remain. Add 10 ml. of 95% ethyl alcohol, 10 ml. of 1*N* hydrochloric acid, and 0.2 ± 0.01 gram of zinc powder. Cover, digest, and evaporate the mixture at low heat to a volume of 5 ml. This takes about 20 minutes. Filter through a 9-cm. Whatman No. 42 filter paper directly into a 25-ml. volumetric flask and wash to a volume of about 20 ml.

Introduce 1 ml. of a 1% sodium nitrite solution, mix, and allow to stand for 10 minutes. Add 1 ml. of a 10% ammonium sulfamate solution, stopper, and invert the flask to remove all the nitrite from the neck of the flask. Allow to stand for 10 minutes with occasional shaking to remove the nitrogen evolved. Add 1 ml. of 1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution, dilute to volume, mix, and allow to stand for 20 minutes.

Read the absorbance on a colorimeter using a 540-mμ filter and obtain micrograms of ONCB by dividing by the absorbance factor determined below. Divide the result by 100 to get parts per million of ONCB in the sample.

Inasmuch as the color follows Beer's law, the absorbance factor giving absorbance per microgram of ONCB may be more conveniently used for calculating instead of employing a standard curve. Run 0- to 30-γ standards in benzene by proceeding at the reduction step using ethyl alcohol, hydrochloric acid, and zinc. Calculate the absorbance per microgram of ONCB for each standard and take the average value. Standard readings obtained on the Evelyn photoelectric colorimeter are given in Table III.

Results and Discussion

Removal of Solvent in Extracts. A serious problem in the determination of volatile organic chemicals is the removal of solvent after extraction without loss of the volatile chemical, particularly when special laboratory equipment is not employed. Table I shows that a complete loss of ONCB occurs when extracts are taken to dryness even by directing a fine stream of air over the surface of the extract in an open beaker at room temperature. Evidently no loss of ONCB occurs if the extract is evaporated to 10 ml. in a covered beaker and the reduction step with zinc and hydrochloric acid, to form *o*-chloroaniline, is carried out in the presence of benzene before diazotization and coupling with *N*-(1-naphthyl)-ethylenediamine. Controls on non-ONCB-treated pineapple fruits have consistently shown negligible amounts of color, indicating that in the presence of benzene and fruit extract, the formation of any aromatic amine compound is unlikely during the reduction step. The absence of appreciable amounts of any naturally occurring aromatic nitro or amine compound is also indicated.

Effect of Acidity During Color Development. In the determination of parathion, Averell and Norris (*1*) specified the use of an amount of hydrochloric acid in excess of that required to react with the quantity of zinc present. The excess must be sufficient to give a

Table I. Effect of Method of Benzene Removal on Loss of ONCB in Extract

Method	ONCB Present, γ	ONCB Found, γ	ONCB Lost, %
Boiled in open beaker to 10 ml., then aired just to dryness	57	0	100
Aired just to dryness	57	3.0	95
Boiled in covered beaker to 10 ml., followed by reduction in presence of benzene	57	56.3	1
Same	34.2	35.4	0

Table II. Effect of Acidity on Development of the Test Color with 34.2 γ of ONCB

Zinc, G.	HCl, Meq.	HCl Excess, Meq., Calcd.[a]	pH	Absorbance	Absorbance/γ/ 25 Ml.[b]
0.2	6	0	1.1	0.441	0.0129
0.1	2.5	0	1.1	0.455	0.0133
0.2	10	3.9	0.7	0.553	0.0162
0.2	10	3.9	0.9	0.561	0.0164

[a] Not directly related to pH, owing to incomplete reaction with zinc.
[b] Obtained on the Evelyn photoelectric colorimeter with filter 540.

Table III. Standard Absorbance Readings Obtained with Filter 540 on the Evelyn Photoelectric Colorimeter

ONCB, γ/25 Ml.	Transmittance, %	Absorbance		A^a/γ
10.7	67	0.174		0.0163
21.4	43¾	0.359		0.0168
32.1	29¾	0.527		0.0164
			Av.	0.0165

[a] A = absorbance.

pH of 0.6 to 1.0 during the coupling reaction in order to achieve maximal color development. Table II gives data on ONCB which confirm this effect. Similar results were obtained on smaller amounts of ONCB. An excess of hydrochloric acid is essential for proper color development.

Adherence to Beer's Law. Absorbance readings obtained on the Evelyn photoelectric colorimeter with filter 540 using matched colorimeter tubes bear a direct linear relationship with varying amounts of ONCB as shown in Table III. The mean absorbance factor, $\frac{\text{Absorbance}}{\gamma}$, is therefore used for the calculations.

Of the various green Evelyn filters available, filter No. 540 is best inasmuch as it transmits light maximally at 540 mμ where maximal absorption of the test color occurs. This is shown by the absorption spectrum in Figure 1 obtained on the Beckman DK-2 spectrophotometer using a 1-cm. cuvette.

Recovery and Sensitivity. Recovery of micro quantities of ONCB added to fresh pineapple fruit tissue and analyzed by the method described above is presented in Table IV. Average recovery of 93% is considered excellent particularly in view of the small amounts of ONCB present.

The method is extremely sensitive—0.01 p.p.m. may be determined. On the Evelyn instrument using the ⅞-inch colorimeter tube, a reading of 96% transmittance is obtained at this concentration.

Literature Cited

(1) Averell, P. R., Norris, M. V., *Anal. Chem.* **20,** 753–6 (1948).
(2) Sherwood, L. V., Monsanto Chemical Co., St. Louis, Mo., private communication, November 8, 1957.
(3) Young, H. Y., Gortner, W. A., *Anal. Chem.* **25,** 800–02 (1953).

Table IV. Recovery of ONCB Added to Pineapple Fruit Tissue[a]

Added, P.P.M.	Recovered, P.P.M.[b]	Recovery, %
0.07	0.06	86
0.07	0.07	100
0.21	0.20	95
0.21	0.19	90
0.36	0.33	92
0.36	0.35	97
	Av.	93

[a] 100-gram sample.
[b] Corrected for a blank value of 0.01 p.p.m. apparent ONCB.

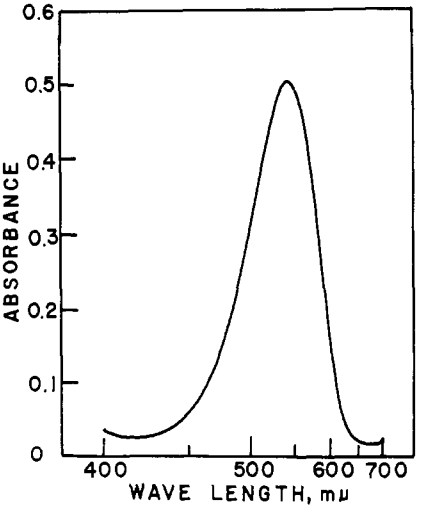

Figure 1. Absorption spectrum of ONCB test color, 55 γ ONCB per 25 ml.

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HERBICIDE RESIDUES

A Colorimetric Method for the Determination of EPTC Residues in Crops and Soils

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ETHYL - *N*,*N* - DI - *n* - PROPYLTHIOLCARBAMATE (EPTC), also known as Eptam, is a promising selective herbicide. A sensitive method which determines the di-*n*-propylamine formed upon hydrolysis of EPTC in concentrated sulfuric acid has been developed for the determination of crop residues. The amine is reacted with carbon disulfide in the presence of ammonia and cupric ion in a two-phase benzene-water system to form, in benzene, the cupric di-*n*-propyldithiocarbamate complex which has an intense absorption peak at 440 mμ. Detection of quantities of EPTC down to 4 γ enables determination of 0.02 p.p.m. of residue by the described procedure.

The colorimetry is based upon the method of Dowden (*1*) for determination of secondary alkylamines, which previously had been adapted to the determination of octamethylpyrophosphoramide (OMPA) by Hall, Stohlman, and Schechter (*3*). OMPA does not interfere, however, when 100 γ are processed through the steam distillation procedure. Possible interference by free amines is eliminated by acidifying the steam distillate prior to extraction of EPTC in preparation for hydrolysis. A wide