Table I. Comparison of Data Calculated by Base-Line Technique vs. Direct Absorption Calculation

Curve	$253^3/_4$ Mμ, P.P.B./Ml.	$283^1/_2$ Mμ, P.P.B./Ml.	Equation 1	Equation 2	Average
A	+2.53	+0.47	+0.33	−0.29	+0.02
B	+2.17	+0.79	+0.11	−0.10	+0.01
C	+2.14	+1.17	−0.02	+0.06	+0.02

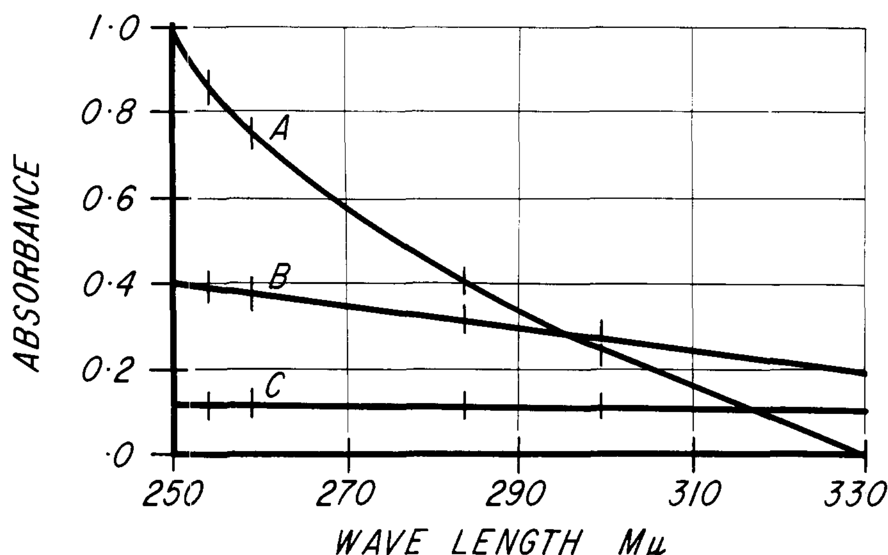

Figure 2. Theoretical spectra of possible interferences caused by the presence of foreign materials in water samples

As the foreign materials that could interfere with the accuracy of this method are unknown, it was necessary to reduce the error due to unknown backgrounds to an absolute minimum.

With the hypothetical spectra of backgrounds as shown in Figure 2, the base-line technique reduced background error more than the absorbance ratio method usually employed in spectrophotometric analyses. The data in Table I show that the base-line method is much less sensitive to foreign materials which may accompany the Phygon into the chloroform extract.

Discussion of Results

As shown in Table II, the Phygon recovery from solutions of known concentrations between 8 to 250 parts per billion is 80% or better. The Phygon is not completely recovered because of two factors: It hydrolyzes slightly even in acid solution, and the steam distillation is not continued long enough to approach 100% recovery.

Solutions of 500 and 750 parts per billion were run, but the recoveries dropped to 70%. The recovery of such high concentration solutions can

Table II. Recoveries of Known Percentages of Phygon in Water between 0 to 250 Parts per Billion

Added, P.P.B.	Recovered, P.P.B.	Recovery, %
0 (Blank)	0.7	...
8.6	7.6	88
10.4	11	106
25	20	80
50	40	80
100	85	85
250	201	80

be improved by collecting a larger distillate and continuing with the normal procedure.

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GROWTH REGULATOR AND HERBICIDE RESIDUES

Extension of the Residue Methods for 1,2-Dihydro-3,6-pyridazinedione (Maleic Hydrazide) and *N*-1-Naphthylphthalamic Acid (Alanap)

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Reported procedures for the determination of 1,2-dihydro-3,6-pyridazinedione [maleic hydrazide (*1, 2*)] (*7*) and *N*-1-naphthylphthalamic acid [Alanap (*4*)] (*5*) are satisfactory when applied to certain food crops at the residue concentrations described. However, interfering components in tobacco preclude the determination of maleic hydrazide residues in this crop by the literature method. Modification of the method is necessary.

The original method has been used successfully in the Naugatuck Chemical laboratories for several years on a wide variety of plant material. It consists of an alkaline digestion of the sample, rapid steam distillation of the sample with zinc as a reductant, and the determination of the hydrazine in the distillate by means of the yellow color formed with *p*-dimethylaminobenzaldehyde.

When maleic hydrazide in amounts up to 200 p.p.m. is added to untreated tobacco, and the tobacco is analyzed by the original procedure given above, a strong red interference coloration is developed, but very little *p*-dimethylaminobenzalazine—the yellow coloration produced by maleic hydrazide.

Basic hydrolysis of maleic hydrazide and determination of the hydrazine formed as described for the determination of maleic hydrazide residues in the literature are not successful on tobacco without modification. Addition of ferrous chloride to the tobacco before caustic distillation gives quantitative recovery of hydrazine. The analytical method is described, and a brief explanation of the use of a base line calculation is presented. A method for Alanap is described in the literature. A revised procedure is given extending the method to determine 0.1 p.p.m. of Alanap on a wider variety of food crops. The method has been applied to peaches, white potatoes, sweet potatoes, cotton plants, peanuts, soybeans, and green beans.

The addition of a small amount of ferrous chloride to the caustic-digested tobacco prior to distillation gave essentially quantitative recovery of maleic hydrazide (Figure 1). This modification was adopted for general use.

A hydrochloric acid precook of the tobacco before distillation from caustic solution also gave essentially quantitative recovery of the maleic hydrazide, but the hydrochloric acid precook was rather time-consuming and sensitive as to the manner of heating and was not adopted for general use.

Initially, the high nitrate ion content of tobacco was assumed to be causing oxidation of the hydrazine liberated from maleic hydrazide before it could distill. Various reducing agents were added to the distillation flask to destroy the nitrate ion. They included the ferrous chloride and hydrochloric acid, mentioned above, which substantially aided recovery, as well as stannous chloride, sodium sulfite, sodium thiosulfate, hydroquinone, and Rubber Antioxidant 2246, which did not aid recovery at all. The material from tobacco which causes the intense red interference coloration was unable to be identified. Experiments showed the following were not instrumental in the low recovery or the red interference coloration. They included sodium nitrite, sodium nitrate, formaldehyde, nitrobenzene, nicotine, and DL-tryptophan.

The procedure of Wood (*7*) for maleic hydrazide residues has been modified in several ways for the analysis of tobacco and a complete description of the revised procedure is necessary.

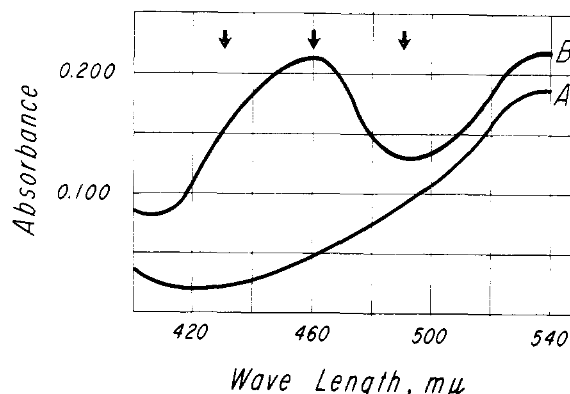


Figure 1. Absorption spectra obtained using ferrous chloride modification

A. Untreated sample
B. Untreated sample + 20 γ of maleic hydrazide

Table I. Recoveries of Maleic Hydrazide Added to Untreated Tobacco

Added, P.P.M.	*Determinations, No.*	*Recovered, P.P.M.*[a]	*Recovery, %*	*Recovery, Av.*
10	2	9.7, 10.0	97, 100	98
30	3	24.3 to 28.5	81 to 95	88
39	1	39	100	100
52	3	45.3 to 52	86 to 100	91
104	4	81 to 97	78 to 92	83
208	1	187	90	90
260	1	216	83	83

[a] Corrected for interference from untreated sample.

Equipment

Maleic hydrazide distillation apparatus (*7*) modified as follows: the 250-ml. distillation flasks, double thickness walls, with thermometer well are superior to the 125-ml. flasks formerly used (*7*). The 250-ml. flasks permit more rapid distillation and reduce foaming problems which have made distillation of some crops, including tobacco, almost impossible in 125-ml. flasks. Available from Macalaster Bicknell Co., New Haven, Conn.

Reagents

Dimethylaminobenzaldehyde reagent. Dissolve 2 grams of *p*-dimethylaminobenzaldehyde in 100 ml. of 1*N* sulfuric acid.

Processing of Sample

Dried tobacco leaf and cigarettes are ground to a dust in a Wiley mill No. 2, equipped with a fine sieve.

Procedure

Add 1.00 ± 0.1 gram of tobacco dust, 50 grams of sodium hydroxide pellets, 40 ml. of distilled water, and 3 to 4 grams of paraffin wax (an excellent antifoaming agent) to a 250-ml. distilling flask. Heat the flask on an electric hot plate to 160° C., determined by a thermometer immersed in oil in the flask's thermometer well. Remove from hot plate, add 0.5 gram of ferrous chloride (analytical reagent grade) and 25 grams (10 ml.) of 30-mesh zinc granules. Quickly grease the socket joint and attach the flask to the distilling apparatus. Heat the flask with a gas flame, receiving the distillate in a 50-ml. graduated centrifuge tube containing 4 ml. of *p*-dimethylaminobenzaldehyde reagent. While the distillation is in process, a nitrogen flow through the apparatus of about two to three bubbles per second breaking from the condenser tip submersed in the reagent is satisfactory for sweeping purposes. When the temperature of the flask's contents has reached 180° C., remove the flame and add approximately 10 ml. of distilled water from the dropping pipet enclosed in the distillation system, and again heat the flask to 180° C. Continue water addition and distillation in this manner until 35 to 40 ml. of distillate are collected.

Remove the receiver from the apparatus and record the volume of distillate obtained. Particles of paraffin wax in the distillate are removed by filtering the distillate through a coarse porosity sintered-glass funnel. Measure the absorbance on a Beckman Model DU spectrophotometer at 430, 460, and 490 mμ in 1-cm. cells, using as reference 4 ml. of the dimethylaminobenzaldehyde reagent diluted to 37 ml. with distilled water.

Calculation

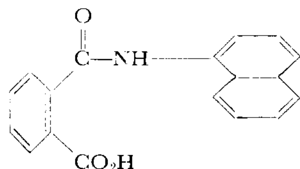
$$\text{P.p.m.} = \frac{5.0\left[\text{absorbance}_{460\ m\mu} - \left(\frac{\text{absorbance}_{430\ m\mu} + \text{absorbance}_{490\ m\mu}}{2}\right) \times \text{ml. of distillate}\right]}{\text{sample weight in grams}}$$

The type of calculation used is the simplest form of the base line technique (*3, 5*). Absorbance measurements at three wave lengths are made, one at the spectrophotometric maximum wave length of the color formed and the other two at wave lengths equidistant from the maximum, where the absorbance measurements of the desired color are considerably less than at the maximum and essentially equal. The average of the absorbances at these two wave lengths gives a valid measure of the background, the magnitude of which may vary according to the conditions of distillation (Figure 1). Such a manner of calculation results in greater accuracy than a simple absorbance difference at one wave length between treated and untreated samples because each analysis is internally compensated for possible variability of intensity of interference, and the red interference in untreated samples gives essentially 0 p.p.m. by use of the base-line technique calculation.

The calibration was obtained by mathematical treatment of spectra (*3, 5*) obtained from various aqueous solutions of purified maleic hydrazide subjected to both the original and the revised procedure over a period of 4 to 5 years. During this period, in approximately 50 analyses on aqueous solutions of purified maleic hydrazide, the deviation of the factor, 5, has been $\pm 5\%$, a rather insignificant contribution to the over-all variability of the method. Analysis of untreated tobacco from various locations showed background interference (in terms of parts per million of maleic hydrazide) varied from $+3$ to -1 p.p.m. Untreated samples to which maleic hydrazide was added, when analyzed by the above procedure, consistently gave satisfactory recoveries as shown in Table I.

DETERMINATION OF ALANAP RESIDUES IN FOOD CROPS

A method is described in the literature for the determination of *N*-1-naphthylphthalamic acid (Alanap) residues in a variety of food crops by the steam distillation of 1-naphthylamine from basic solution and reaction with diazotized sulfanilic acid (*5*).



N-1-naphthylphthalamic acid (Alanap)

This method is successful on a wide variety of food products when the residual Alanap is approximately 4 p.p.m. As its uses were extended and lower residue levels were sought, the method was not adequate.

Major changes have been made in the original analytical method, in order to determine residues at the 0.1-p.p.m. level. The sample size has been increased considerably, as has also the magnitude of the interferences. The interferences, however, are eliminated after *n*-hexane has selectively extracted the 1-naphthylamine from the distillate.

The 1-naphthylamine is then extracted from the *n*-hexane into aqueous acid. Addition of diazotized sulfanilic acid to the aqueous acid develops a red coloration with 1-naphthylamine and a very weak yellow coloration with the small amount of residual interfering compounds present.

Measurement of the color at three wave lengths and calculation by the base line technique, discussed at length by Newell, Mazaika, and Cook (*3*), minimizes the variation caused by the remaining interferences. The revised method is given below.

Equipment

Round-bottomed flask, 5-liter, with 45/50 ₸ joint.

Reducing adapter with top 24/40 outer and bottom 45/50 inner ₸ joint.

Kjeldahl cylindrical connecting bulb with 24/40 inner ₸ joints on both ends.

West-type condenser, 300-ml. jacket length with top 24/40 outer and bottom 24/40 inner ₸ joints.

105°-connecting tube with suction tube (170-mm. stem length) having a top outer 24/40 inner ₸ joint.

Reagent

Sulfanilic acid, 1% in 30% acetic acid–water solution (prepared by heating sulfanilic acid in 30% acetic acid solution).

Procedure

A 200-gram sample of the ground material is placed in a 5-liter, round-bottomed flask with 1500 ml. of 30% sodium hydroxide solution, 5 grams of paraffin wax, 2 ml. of Dow-Corning Antifoam A, and 1 gram of 20-mesh zinc.

Zinc is added to the distilling flask, to protect the α-naphthylamine from being oxidized by components present in the sample. Without zinc addition, recoveries of 0.1 p.p.m. of Alanap are low.

Paraffin wax and Antifoam A in combination was the most successful antifoam agent for use in the distillation of all dried plants, seeds, and nuts from caustic solution. No antifoam agents are needed in the distillation of peaches and sweet and white potatoes.

The flask is swirled to wet the sample thoroughly then set in a heating mantle. The remaining pieces of the distilling apparatus are joined tightly—each clean, dry, joint is lubricated with glycerol before joining. Set a 500-ml. iodine flask as the receiver, so that the long stem of the connecting tube goes to the bottom. With rapid condenser water flow, initiate heating with a Variac setting of about 120. When an inch or so of foam has formed on the surface of the flask's contents, set the Variac at 70 until distillation begins. If the foaming should climb to the neck of the flask, a cold, wet cloth placed on the exposed surface of the flask will reduce it in a few seconds. In a short time, the foaming will remain constantly at a low level. Heating may be increased slowly, until a Variac setting of 100 to 110 is reached, which will give a rapid distillation.

When 200 ml. of distillate is obtained, transfer the distillate from the iodine flask to a 500-ml. separatory funnel. Rinse the condenser, connecting tube, and iodine flask, one after the other, with a single 100 ml. of *n*-hexane, transferring the hexane to the separatory funnel. Shake the funnel briskly for 1 minute, allow the layers to settle for 2 minutes, then draw off and discard the aqueous layer. Wash the hexane layer with five 100-ml. portions of distilled water, shaking each wash briskly with the hexane layer and allowing a settling period of 2 minutes before drawing off and discarding each wash. The water washings remove essentially all the remaining potential interferences.

To the washed hexane layer in the separatory funnel, add 4.5 ml. of distilled water and one drop of concentrated hydrochloric acid. Shake the funnel thoroughly for 1 minute. Test the wet, ground glass stopper for pH of aqueous phase with 0.0- to 1.5-pH range Hydrion paper to assure a pH of 1.0 or below. If pH is higher, add a second drop of concentrated hydrochloric acid and shake thoroughly. Let the solution stand for 2 minutes before drawing the aqueous layer into a 25-ml. mixing cylinder. At this point, samples may be stored for as long as 24 hours before developing the color.

To the acid solution in the mixing cylinder, add two drops of 30% sodium hydroxide for each drop of concentrated hydrochloric acid added above and mix thoroughly. Then, add 10 drops of coupling agent. Prepare the coupling agent fresh each time it is needed, by mixing equal volumes of 1% sulfanilic acid solution and 0.12% sodium nitrite solution, 5 to 30 minutes before use. Dilute the solution in the graduated cylinder to 10 ml. with con-

centrated acetic acid and measure the color in 1-cm. Corex cells on a Beckman Model DU spectrophotometer at 480, 534, and 600 mμ wave length setting one-half hour after addition of the coupling agent.

Calculations

Cotton, Soybeans, and Peanuts. It was necessary to derive an equation from a series of check samples, using the geometry of the curves combined with that curve of the desired constituent so that consistent recovery data could be obtained. The interference curve moves up or down (Figure 2) depending on rate of distillation, efficiency of extraction, and other factors. Under these conditions, the base line technique of calculation was particularly valuable, and the mathematics of such derivations are discussed by Newell, Mazaika, and Cook (*3*) and Tunnicliff, Rasmussen, and Morse (*6*).

Table II shows actual recovery data from a series of individual untreated samples, to which 0.10 p.p.m. of Alanap had been added before distillation. They include above-the-ground parts of whole mature cotton plants, soybean plants, and whole peanut plants with roots and nuts, peanut hay, and ground whole peanuts with shucks. From 13 determinations of untreated plants, nuts, and seeds, interference in terms of parts per million of Alanap has averaged 0.00 with a high of +0.03 and a low of −0.04.

$$\text{P.p.m.} = \frac{640\ (\text{absorbance}_{534\ m\mu}) - 288\ (\text{absorbance}_{600\ m\mu}) - 352\ (\text{absorbance}_{480\ m\mu})}{\text{sample weight in grams}}$$

Cottonseed Meal. Recoveries of knowns, from ground cottonseed, using the described procedure, were variable and somewhat low. Increasing the zinc from 1 to 25 grams gave more consistent and more quantitative data. The use of 25 grams of zinc also has produced somewhat greater color intensity for a given amount of Alanap, than was obtained with 1 gram of zinc. However, there was no difference in interference level or spectrum. Thus, for ground cottonseed, when 25 grams of zinc were used, the above equation was modified to account for the increased color intensity obtained from Alanap. The modified equation is given below:

$$\text{P.p.m.} = \frac{434\ \text{absorbance}_{534\ m\mu} - 195.2\ \text{absorbance}_{600\ m\mu} - 238\ \text{absorbance}_{480\ m\mu}}{\text{sample weight in grams}}$$

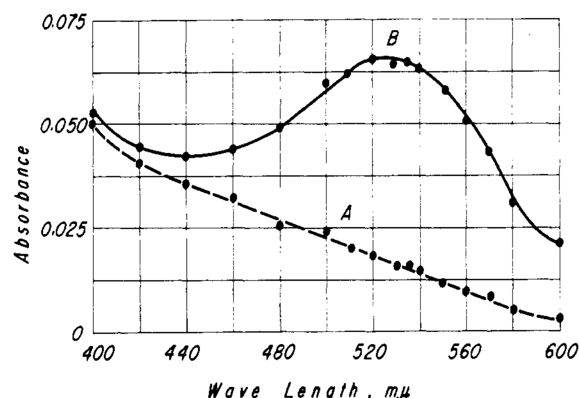


Figure 2. Absorption spectra using improved method

A. Untreated soybeans
B. Untreated soybeans + 15 γ of Alanap

Table II. Recoveries of Alanap Added to Untreated Food Crops

Crop	Added, P.P.M.	Determinations, No.	Recovered, P.P.M.[a]	Recovery, %	Recovery, Av.
Beans	0.08	3	0.06 to 0.08	78 to 98	91
Melons	0.10	1	0.073	73	73
Peaches	0.10	2	0.08 to 0.10	80 to 100	90
Potatoes					
Sweet	0.10	5	0.07 to 0.13	70 to 130	88
White	0.10	3	0.09 to 0.114	90 to 114	95
	0.20	1	0.18	90	90
Cottonseed	0.10	4	0.06 to 0.12	60 to 120	83
(+25 grams zinc)	0.10	4	0.07 to 0.11	70 to 110	90
Peanut vines	0.10	1	0.08	80	80
Soybeans	0.10	4	0.089 to 0.11	89 to 110	100

[a] Corrected for interference from untreated samples.

Peaches and Sweet and White Potatoes. For the analysis of peaches and sweet and white potatoes, the calculation used is a simpler form of the base line technique than was used above. Interference coloration from these fleshy crops is essentially nil in the spectral range used for measurements. From 12 determinations of untreated fleshy crops, interference in terms of parts per million of Alanap has averaged 0.00—with a high of 0.00 and a low of −0.01.

$$\text{P.p.m.} = \frac{\left[\text{absorbance}_{534\ m\mu} - \left(\frac{\text{absorbance}_{480\ m\mu} + \text{absorbance}_{600\ m\mu}}{2}\right)\right]}{\text{sample weight in grams}}\ 323$$

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