# Determination of Naphthaleneacetic Acid in Apples

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For the determination of naphthaleneacetic acid (NAA) in apples, the compound is extracted with dilute alkali and separated from interfering materials by partition chromatography on a silicic acid column. Both ultraviolet absorption at 283 mμ and a colorimetric procedure are used for measuring residues. The ultraviolet method is sensitivite to 0.1 p.p.m., and recoveries averaged 93.5%. The colorimetric procedure is sensitive to 0.2 p.p.m., and recoveries averaged 79.1%. Residues of the chemical were less than 0.1 p.p.m. 1 hour after application of a spray containing 20 p.p.m. of active ingredient.

NAPHTHALENEACETIC ACID (NAA) has been used for 20 years in apple orchards for control of preharvest drop (*2*), and for 15 years as a thinning spray for apples, applied shortly after full bloom (*5*). It has become increasingly important to have an analytical procedure for detecting NAA in fruits and leaves. Possibilities of using a spectrophotometric method for analysis of NAA residues in the fruit have been studied.

Luckwill (*3*) extracted NAA from tree leaves with dilute ammonia. Bhargava and Heidelberger (*1*) presented a method for the partition chromatographic separation of aromatic acids including NAA. Schwartz and Kuramoto (*6*) determined NAA as one of the hydrolysis products of naphazoline by ultraviolet absorption at 283 mμ with chloroform as a solvent. Zweig (*7*) determined NAA in potatoes by a method involving gas chromatography and quantitative measurement by ultraviolet absorption.

The following procedure involves extraction with dilute ammonia, separation of NAA on a silicic acid column, and two methods of quantitative measurement of the acid. One method involves ultraviolet absorption at 283 mμ, and the other, the reaction of NAA with $NaNO_3$ in concentrated $H_3PO_4$ to produce a yellow color that is measured at 370 mμ.

The temporary tolerance set for NAA in 1950 is 1 p.p.m. based on the expected toxicity of the compound. As evidenced by residue data presented, the colorimetric procedure is entirely adequate to measure residues down to 0.2 p.p.m. For those workers who do not have readily available to them a spectrophotometer capable of making ultraviolet measurements, or because of the nature of their work do not choose to work in the ultraviolet range, a colorimetric method could be very desirable. This color method could also be of value in laboratories working with other crops where no color test for NAA exists. The two methods of analysis for the same substance could be used for comparative studies.

## Procedure

**Extraction.** Blend portions of several apples totaling 100 grams with 250 ml. of 0.1*N* $NH_4OH$ for 3 minutes. Add 25 grams of Celite 545 and continue blending for 1 minute more. Filter the slurry through S&S 595 paper covered with 10 grams of Celite 545 in a Büchner funnel. After filtration, return the apple pulp to the blender together with 250 ml. more of 0.1*N* $NH_4OH$ and blend again for about 2 minutes. Filter through a filter prepared as above. Wash the filter and pulp with three 100-ml. portions of distilled water. Combine the aqueous extracts and water washings, and add 25 grams of Celite 545. While stirring vigorously with a magnetic stirrer, add 15 ml. of concentrated phosphoric acid and continue stirring for about 5 minutes. Filter through S&S 595 paper covered with 10 grams of Celite 545, and wash the filter pad with two 100-ml. portions of water. Combine the dilute acid extracts and water washings, and extract with 400 ml. of diethyl ether in a continuous liquid-liquid extractor for 8 hours. This extraction can also be done in a large separatory funnel by shaking successively with 200, and three 100-ml. portions of diethyl ether. In warm weather, however, the vapor pressure of ether makes the use of the extractors more desirable. Wash the ether extracts three times with 25 ml. of water and discard the water washings. Evaporate the ether extracts to dryness.

**Chromatography.** Further purify silicic acid previously washed free of fines by washing twice with concentrated HCl as in the method of Resnik *et al.* (*4*), and once with 5*N* HCl, and then washing free of chlorides with distilled water to pH 5.0. The silicic acid is dried for 24 hours in an oven at 110° C. with occasional stirring. The dried acid is aged in a tight container for 3 weeks before use. The chromatographic column consists of a borosilicate glass tube, 15 mm. o.d. and 200 mm. long, with a 100-ml. reservoir attached to the top. A constriction at the bottom filled with glass wool supports the column packing. Equilibrate 500 ml. of redistilled Skellysolve B with 100 ml. of 9:1 aqueous methanol (90%)–0.5*N* $H_2SO_4$. The upper layer is used as the mobile phase and the lower layer as the stationary phase. Ten grams of the silicic acid are hydrated with 7 ml. of stationary phase added in three portions. Add a slurry of 7 grams of the hydrated silicic acid in 45 ml. of mobile phase to the column. Allow the column to settle and then pack with enough air pressure to allow a column flow of about 2 ml. per minute (usually about 3 to 4 pounds). Place a small disk of Whatman No. 1 paper on top of the column packing and allow the solvent level to run down just to the filter paper. Dissolve the sample completely in 0.25 ml. of ethyl acetate and then add 2 ml. of the mobile phase. Some precipitation will occur, but the NAA will not precipitate. Using a small pipet, add the dissolved sample to the column, and rinse the beaker with 1 ml. of the mobile phase, and add this to the column. Using air pressure, drain the solvent level down to the filter paper. Add three 1-ml. portions of the mobile phase, draining each portion to the level of the filter paper. Elute the column with 110 ml. of the mobile phase. Discard the first 30 ml. of the eluate. Collect the next 10 ml., the following 50 ml., and the final 10 ml. as separate fractions. Evaporate each fraction to dryness separately on a steam bath.

The three fractions were collected to serve as a check on the separation or elution pattern of the column. The silicic acid is hydrated, and the solvent systems are equilibrated just prior to use for each set of analyses. Small

**Table I. Recoveries of NAA from Apples**

| Added, P.P.M. | % Recovery 283 mμ | 370 mμ |
|---|---|---|
| 0.15 | 102.7 | 64.7 |
| 0.15 | 90.7 | ... |
| 0.25 | 91.2 | 75.6 |
| 0.25 | 85.2 | 89.6 |
| 0.50 | 99.0 | 85.6 |
| 0.75 | 92.0 | 80.1 |

differences in these steps of the method will produce correspondingly small differences in the column separation. Adoption of this technique makes it possible to collect the NAA in the smallest practical volume of eluate, and the check values are also kept to a minimum. In the experiment described, all of the NAA was contained in the 50-ml. fraction except in the case of a single pair of samples and corresponding check and recovery. In that instance, where a small amount of NAA appeared in the last 10-ml. fraction, the values obtained for both fractions were added together.

**Analysis.** Ultraviolet. Dissolve each fraction of each sample in 5 ml. of $CHCl_3$. Measure the absorbance of these solutions with a Beckman DU spectrophotometer in a 1-cm. cell at 283 mμ with $CHCl_3$ as a reference.

Colorimetric. Add 5 ml. of 0.1% $NaNO_3$ in concentrated $H_3PO_4$ (prepare by heating to dissolve the salt) to the evaporated residue of each fraction. Nitrate the fractions by heating on a steam bath with occasional swirling for 10 minutes. Allow the samples to cool about 15 minutes, and read at 370 mμ in a 1-cm. cell using the $NaNO_3$-$H_3PO_4$ solution as a reference. The yellow color is stable for at least 2 hours. A check and recovery are run with each pair of samples.

**Standard Curve.** Pipet 0, 2, 5, 10, and 15 ml. of a standard NAA solution containing 5 μg. per ml. into a series of 50-ml. beakers and make up to 15 ml. with water. Evaporate to dryness on a steam bath and analyze as described for the samples. This technique corrects for any error introduced by the addition of different volumes of water. Any losses caused by evaporation at 100° C. would also be corrected for.

**Table II. Analysis of Field Treated Samples**

| Harvest Date | Interval | Residue, P.P.M. 283 mμ | 370 mμ |
|---|---|---|---|
| 20 P.P.M. Application | | | |
| 9/19[a] | 1 hr. | 0.0 | 0.0 |
| | | 0.0 | 0.1 |
| 9/20 | 24 hr. | 0.0 | 0.0 |
| | | 0.1 | 0.1 |
| 9/29 | 10 days | 0.0 | 0.0 |
| | | 0.0 | 0.0 |
| 200 P.P.M. Application | | | |
| 9/26[a] | 1 hr. | 0.5 | 0.3 |
| | | 0.6 | 0.2 |
| 9/27 | 24 hr. | 1.2 | 1.1 |
| | | 0.8 | 0.7 |
| 10/3 | 7 days | 0.0 | 0.1 |
| | | 0.0 | 0.1 |

[a] Application date.

### Results and Discussion

Apple trees were treated with the normal spray concentration of 20 p.p.m. for drop control. A separate tree was sprayed at 10 times the normal rate or 200 p.p.m. to measure the disappearance of NAA from the fruit more accurately.

Table I shows recoveries of NAA added to fruit before extraction. For the ultraviolet determination, checks averaged 0.095 p.p.m. with a standard deviation of 0.032. For the colorimetric determination, checks averaged 0.134 p.p.m. with a standard deviation of 0.024.

Table II shows the results of these two experiments. Where 20 p.p.m. of NAA was applied, the residue after 1 hour is less than 0.1 p.p.m. Where 200 p.p.m. was applied, in 7 days the residue of NAA had declined to below 0.1 p.p.m.

### Literature Cited


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**INSECTICIDE METABOLISM**

# The Detection of Dieldrin Metabolites in Human Urine


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Dieldrin (1,2,3,4,10,10-hexachloro-6,7 - epoxy - 1,4,4a,5,6,7,8,8a-octahydro - 1,4-*endo-exo* - 5,8 - dimethanonaphthalene) has found extensive use in this country and in many other areas of the world as a means of controlling insects of both agricultural and communicable disease importance. The toxicity of the insecticide to spraymen (*10*) and the limited knowledge of its storage (*2, 15*) and elimination (*8*) by humans indicate a need for further studies on the metabolic fate of the compound.

The conversion of aldrin (1,2,3,4,10,10-hexachloro-1,4,,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene) to dieldrin in mammals was first observed by Barnes as reported by Winteringham and Barnes (*16*), and the conversion was later confirmed by others (*1*). Using houseflies, Brooks (*5*) not only demonstrated the metabolism of aldrin to dieldrin and isodrin to endrin, but also obtained data that indicated further degradation of endrin to its known keto rearrangement product (*3*). Indication of the possible metabolic breakdown of dieldrin itself was reported as early as 1953 by Kunze and Laug (*13*). Using a bioassay technique, these workers were able to detect a toxic material different from the parent compound in both the kidneys and the urine of male rats fed dieldrin in their diets. Further details of this work have not been published. Winteringham and Harrison in 1959 (*17*), using a topical application of sulfur-35 analog of dieldrin on houseflies, detected unidentified metabolites of the compound by paper chromatography in both the body and the excreta of the flies.

In the early work on the toxicity of dieldrin to spraymen, Blazquez and Bianchini (*4*), using a bioassay technique, determined the presence of a toxic material in the blood of men exposed to dieldrin and speculated on the possible elimination of this compound in the bile, feces, and urine. Fletcher (*8*), using the knowledge obtained from investigations on the storage of DDT and the elimination of DDA in man (*12*), determined, by the total