# Colorimetric Determination of Alar Residues in Apples

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A colorimetric method for the determination of Alar residues in apples with a sensitivity of about 1.5 p.p.m. has been developed. The method involves alkaline extraction and hydrolysis of Alar to dimethylhydrazine and steam distillation of the latter. Dimethylhydrazine is determined colorimetrically based on its reduction of phosphomolybdic acid to the heteropoly blue. Recovery of Alar from fruit at levels from 2.5 to 10 p.p.m. ranged from 68 to 114%. Analysis of orchard-sprayed fruit showed harvest residues which ranged from less than 1.5 to 48.5 p.p.m. of Alar depending on the spray concentration (500 to 5000 p.p.m.), number of applications (1 to 3), and interval (1 to 4 months).

Alar (*N*-dimethylaminosuccinamic acid) is an effective growth retardant in the culture of apple, cherry, and pear trees (Batjer *et al.*, 1964). The use potential of this compound prompted investigations to evaluate various application procedures and to correlate these with resultant fruit residues at harvest. In the work reported, a newly developed colorimetric method was used to measure Alar residues in apples as a function of time and rate of application during two growing seasons.

## METHODS AND MATERIALS

Alar was applied as a foliar spray to several varieties of mature apple trees at the Cornell orchard in Ithaca, N.Y. Each test consisted of a single spray application made at a specific time between May and September. Alar formulated at concentrations from 500 to 5000 p.p.m. were tested. Fruit was harvested and determination of Alar residues followed.

The determination of Alar in apples is a modification of the method of Browning (1963), involving alkaline extraction and hydrolysis of Alar to dimethylhydrazine, and steam distillation of the latter. Dimethylhydrazine was determined colorimetrically based on its reduction of phosphomolybdic acid to the heteropoly blue. This is just the reverse of the familiar use of hydrazine salts to reduce heteropoly acids (Kingsley and Shaffert, 1951). The procedure was as follows: Several (15 to 20) apples were chopped in a food chopper and thoroughly blended into a uniform puree. Ten grams were subsampled for extraction and placed in a 50-ml. beaker with 20 ml. of 50% sodium hydroxide. The contents were mixed, and the container was covered and allowed to stand overnight at room temperature. The mixture was filtered through two layers of cheesecloth into a 250-ml. Erlenmeyer flask with 24/40 standard taper joint. The contents of the beaker were rinsed with 10 ml. of 60% sodium hydroxide and the rinse solution was transferred to the cheesecloth. The cloth was squeezed to remove remaining liquid, and this was drained into the Erlenmeyer flask.

The condenser (Batchelder and Patchett, 1958) (Figure 1) was then attached and the solution heated on a Lindberg hot plate until 8 ml. of distillate were collected in a 15-ml. graduated centrifuge tube. Exactly 1 ml. of 5% phosphomolybdic acid was added to the tube, and the contents were heated in a boiling water bath for 1 hour. The contents were cooled and made to 10 ml. with distilled water, and the absorbance was determined at 625 mμ in a 4-cm. cell. If the colored solution was turbid, it was centrifuged prior to measurement.

The standard curve (0 to 100 μg. of Alar) was developed as follows: 0, 1, 2, 3, and 4 ml. of aqueous standard Alar solution (25 μg. per ml.) were transferred to each of five 250-ml. Erlenmeyer distillation flasks. Water was added to a total volume of 4 ml. in each;

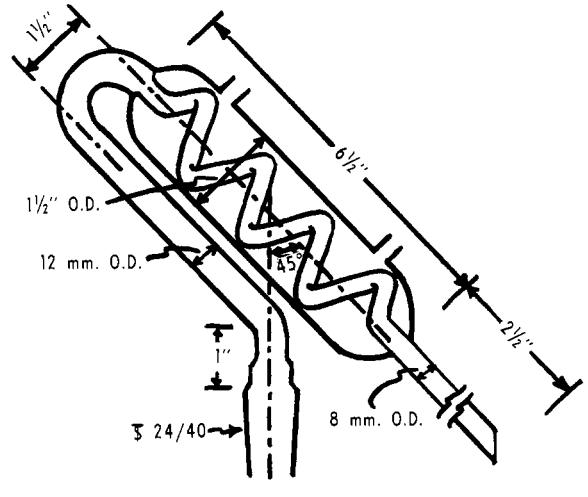


**Figure 1. Alar steam distillation head**

**Table I. Recovery of Alar from Apples**

| Variety | Alar Added, P.P.M. | Recovery, % |
|---|---|---|
| Cortland | 2.5 | 76, 68 |
| Idared | 5 | 108 |
| McIntosh | 2.5 | 86, 106, 104, 78, 114, 91, 135, 96, 80 |
| | 5 | 86 |
| | 10 | 83 |
| Red Delicious | 5 | 106 |

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Table II. Residues of Alar in Apples from Field-Sprayed Trees

| Variety | Alar Spray Concentration, P.P.M. | Application Date(s) | Harvest Date | Alar Residue, P.P.M. (Corrected for Control) |
|---|---|---|---|---|
| | | 1964 | | |
| Cortland | 2000 | June 5 | Oct. 3 | 21.4 |
| McIntosh | 500 | Aug. 29 | Oct. 3 | 1.6 |
| | 500 | Sept. 17 | Oct. 3 | 3.0 |
| | 1000 | Aug. 29 | Oct. 3 | 13.2 |
| | 2000 | Aug. 29 | Oct. 3 | 33.5 |
| | 2000 | Sept. 17 | Oct. 3 | 37.7 |
| | 2000 | Sept. 17 | Oct. 3 | 30.9 |
| | 2500 | May 8 | Oct. 3 | 4.3 |
| | 2500 | May 22 | Oct. 3 | 3.1 |
| | 2500 | July 1 | Oct. 3 | 4.3 |
| | 2500 | Aug. 1 | Oct. 3 | 48.5 |
| | 2500 | Sept. 1 | Oct. 3 | 10.1 |
| | 2500 | Sept. 21 | Oct. 3 | 17.2 |
| R. I. Greening | 1000 | June 23 | Oct. 3 | 5.4 |
| | 4000 | Sept. 16 | Oct. 3 | 36.5 |
| | 5000 | June 23, 1966 | Oct. 3 | 38.9 |
| Cortland | 500 | June 1964, 1965, 1966 | Oct. 17 | 1.7 |
| | 2000 | June 1964, 1965, 1966 | Oct. 17 | 5.1 |
| Idared | 1000 | June 1964, 1965, 1966 | Oct. 29 | <1.5 |
| | 2000 | June 1964, 1965, 1966 | Oct. 29 | 3.0 |
| McIntosh | 2000 | Aug. 29, 1964 | Sept. 7 | <1.5 |
| | 500 | June 1964, 1965, 1966 | Sept. 27 | 2.5 |
| | 2000 | June 1964, 1965, 1966 | Sept. 27 | 3.4 |
| | 500 | Aug. 1, 1965 and July 26, 1966 | Oct. 5 | <1.5 |
| | 1000 | Aug. 1, 1965 and July 26, 1966 | Oct. 5 | 2.3 |
| | 2000 | Aug. 1, 1965 and July 26, 1966 | Oct. 5 | 8.7 |
| | 500 | Aug. 23, 1966 | Sept. 7 | 2.5 |
| | 1000 | Aug. 23, 1966 | Sept. 7 | 5.4 |
| Red Delicious | 2000 | June 1964, 1965, 1966 | Oct. 29 | 8.6 |

30 ml. of 50% sodium hydroxide were added, the condensers were attached, and the standards were then treated as in the analysis of samples.

## RESULTS

The recovery of Alar added to several varieties of apples before alkaline extraction is listed in Table I. The magnitude of "apparent Alar" in control fruit ranged from 1.6 to 2.5 p.p.m. The method will detect about 1.5 p.p.m. of Alar.

The analyses of field-treated samples during two seasons are presented in Table II. The residues found at harvest agree well with the time, concentration, and number of spray applications. The 500-, 1000-, and 2000-p.p.m. spray concentrations applied between July 15 and August 1 virtually always gave the desired growth effects. The corresponding harvest residues ranged from 3.3 to 11.2 p.p.m. Owing to the low mammalian toxicity of Alar (the acute oral toxicity for rats is 8400 mg. per kg.), the manufacturer is seeking a tolerance of 30 p.p.m., which would constitute a toxicologically safe and reasonable level of Alar to be legally permitted on marketed fruit.

## LITERATURE CITED


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