# Analysis of Amitrole–Simazine Formulations

A simple method was developed for analysis of amitrole–simazine formulations. The 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine) is quantitated by gas chromatography, and the 3-amino-1,2,4-triazole (amitrole) is quantitated by measuring the absorbance of the amitrole–nitroprusside complex at 632 nm according to the method of Sund (1956).

The analysis of amitrole–simazine mixed formulations (suspensions of simazine in solutions of amitrole) has caused problems in this laboratory. The Official Method of Analysis (AOAC, 1970) for amitrole involves a base titration which is inherently nonspecific and requires determination of an inflection point.

Simazine analysis of these formulations is also difficult, since the simazine is present as a suspension, thereby leading to sampling problems. The two recommended methods of analysis (Knusli, 1964) require titrations which are nonspecific and subject to interferences.

Amitrole standard solutions may be satisfactorily chromatographed by glc on glass columns, but not on stainless steel columns. However, gas chromatography of amitrole formulations does not yield an amitrole peak. This is probably due to its reaction with most acids and bases, and to its chelating properties with metallic salts and cations (Spencer, 1968; Sutherland, 1964), which are usually present in amitrole–simazine formulations. A simplified sample preparation is developed to quantitate amitrole (by spectrophotometry) and simazine (by gas chromatography) without prior removal of interfering substances.

## EXPERIMENTAL SECTION

**Sample Preparation.** It is necessary to shake the amitrole–simazine formulations well prior to weighing samples. It is also necessary to pour the samples rather than pipet them to ensure quantitative transfer of the suspension. These precautions will facilitate sampling in the field, and the possibility of error in sampling can be avoided by obtaining the entire commercial container of samples.

Pour a predetermined amount of shaken sample containing less than 0.04 g of simazine (usually 1–2 ml) into a tared 100-ml volumetric flask and reweigh. Add methanol to make to volume, shake for 30 sec, and then let stand for at least 72 hr.

In the preparation of simazine standard and amitrole–simazine sample solutions, it is important to keep the concentration of simazine below the 400-ppm (400 mg/l.) level, which is the solubility of this herbicide in methanol at 20°. Shake all solutions immediately prior to using.

**Simazine Analysis.** Prepare the standard by dissolving less than 0.1 g of technical standard (97.2%) in 250 ml of methanol.

The gas chromatographic analysis was carried out using a Pye 104 instrument equipped with a flame ionization detector under the following conditions: borosilicate glass column, 6 ft × 4 mm i.d.; 3% Carbowax 20M on 80/100 mesh Gas Chrom Q; injection port, 270°; detector, 280°; column, 230°; nitrogen, 75 ml/min; injection volume, 5 $\mu$l; and retention time, 1.06 min. (Other columns and temperatures used successfully were 3% OV-7 on 80/100 mesh Chromosorb W H.P. at 190° and 3% OV-225 on 80/100 mesh Chromosorb W H.P. at 230°.) Dilute if necessary to obtain similar peak heights. Measure the peak heights and calculate the percent simazine in the samples. Convert the percentages to appropriate units.

**Amitrole Analysis.** Amitrole is estimated according to the method of Sund (1956) by measuring the absorbance of the amitrole–nitroprusside complex.

The nitroprusside reagent is prepared from the following reagents: (a) dissolve 42.2 g of potassium ferrocyanide trihydrate [$K_4Fe(CN)_6 \cdot 3H_2O$] in distilled water and dilute to 500 ml in a low actinic volumetric flask; (b) dissolve 29.8 g of sodium nitroprusside dihydrate [$Na_2Fe(CN)_5NO \cdot 2H_2O$] in distilled water and dilute to 500 ml in a low actinic volumetric flask; (c) 10% sodium hydroxide; and (d) 3% hydrogen peroxide (prepared when required from 30% $H_2O_2$).

Mix the solutions (a), (b), (c), and (d) in the proportion of 2:2:1:5 and let stand 15 min. Then add 1.2 ml of glacial acetic acid per 100 ml of solution. NOTE: This reagent must be prepared daily. Prepare the standard by dissolving about 0.2 g of technical standard of amitrole (mp 152–154°) in 250 ml of methanol.

Into three 100-ml volumetric flasks add separately 25 ml of methanol (blank), 5 ml of amitrole standard and 20 ml of methanol, and 25 ml of sample (or appropriate dilution with methanol). To each 100-ml volumetric flask, add 25 ml water, 3 drops of 10% sodium hydroxide, and 10 ml of nitroprusside reagent. Bring each solution to volume with distilled water, shake briefly, and let stand for 3 hr. Pipet and then gravity-filter 5-ml aliquots. Measure the absorbance of the aliquots at 632 nm on a Beckman DU with a slit width of 0.05 mm. (Other spectrophotometers may be used.)

Calculate the percent amitrole in the samples and convert to appropriate units.

## RESULTS AND DISCUSSION

Solutions of simazine at its solubility limit and nitroprusside reagent gave no absorbance at 632 nm, so there is no interference with the amitrole analysis. The absorbance of the amitrole–nitroprusside complex was found to be linear over the range 2–6 mg of amitrole (0.200–0.900 absorbance units). To ensure linearity of simazine results, the peak height of the standard should be matched to within 20% of the sample peak height.

The 72-hr waiting period during the sample preparation could, perhaps, be shortened, but this would involve more working time for the analyst and there are problems in stirring these formulations which lead to resinous material in methanol.

The above amitrole method may also be used for the analysis of aqueous amitrole formulations with the following changes. The sample is prepared by pipetting aliquots, and distilled water is used as solvent for samples and standards. The sample solution may be analyzed immediately. Appropriate concentrations must be used.

Typical results from triplicate determinations on two different amitrole–simazine formulations are as given in Table I.

**Table I**

| | Amitrole | | Simazine | |
|---|---|---|---|---|
| | A | B | A | B |
| Mean percentage | 1.11 | 5.47 | 2.99 | 9.97 |
| Standard deviation | 0.01 | 0.05 | 0.06 | 0.06 |
| Relative standard deviation | 0.9% | 0.9% | 2.0% | 0.6% |

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# Residue Studies of *O,S*-Dimethyl Phosphoroamidothioate on Tomatoes

Residues of *O,S*-dimethyl phosphoroamidothioate were determined in mature tomatoes. Residue values were obtained as a function of the insecticide doses and the time elapsed between final treatment and harvesting time. Plants treated with multiple doses of 0.5 kg of active ingredients/ha and 1.0 kg of active ingredients/ha yielded residues of 0.19 and 0.84 ppm, respectively, when harvested 18 days after the final treatment. Plants receiving one single treatment of 0.5 kg of active ingredient/ha yielded residues between 0.072–0.013 ppm.

The compound *O,S*-dimethyl phosphoroamidothioate (Tamarón, Bayer; Monitor, Chevron Chemical Co.) is an insecticide-acaricide of systemic activity which also is effective by contact action in controlling a variety of insects in several crops such as cotton, tomatoes, barley, corn, and apples. This organophosphorus compound is extensively sold in Central and South America and Africa. No published data exist as to the residue levels of this insecticide in plants. Thus, it was the purpose of this work to find the residue levels in tomato fruits by varying the dose of the applied material and the time interval between the last treatment and harvesting time. At present, there are no established residue levels for this organophosphorus insecticide.

## EXPERIMENTAL SECTION

**Plant Treatments.** Plants of the V. F. *Napoli* variety were grown in seedbeds at El Cortijo, Villa de Cura, and after 25 days they were transplanted in an experimental field at the Shell Foundation Experimental Station located in Cagua, Estado Aragua. On December 17, 1971, plants were transplanted at 0.5-m intervals in rows spaced at 1.5 m. The field was divided into two sections. One section received one single treatment of 0.50 kg of active ingredient/ha 2, 4, 6, and 8 weeks prior to harvesting. Plots consisted of 3 rows, each 3 m long, having an area of 4.5 $m^2$. Each plot was separated from the next one by a single untreated row. The plants in the second section were subdivided into two groups which received four doses of 0.50 and 1.0 kg of active ingredient/ha, respectively, at weekly intervals. Each plot consisted of 5 rows, 5 m long, having an area of 37.5 $m^2$. Multiple treatments were applied on the 4, 11, 18, and 28 of February 1972. Insecticide was applied with a mist blower calibrated to deliver 420 l./ha. The total number of plants used was 519, which were planted in an area of 925 $m^2$. A control plot similar to those previously described, in a neighboring area, was planted and those plants did not receive insecticide treatment. Plants were given all the conventional agronomical practices such as irrigation, fertilization, deweeding, etc. During the experiment 26.4 mm of rain were collected on January 29, 1972. The tomato plants which received one single treatment were harvested on March 17, 1972. The plants which received multiple treatments were harvested at 1, 3, 7, 10, 14, and 18 days after the last insecticide treatment.

**Analytical Procedure.** Samples of about 1–2 kg were collected for each analysis and duplicate runs were made. About 8–10 unwashed tomatoes were quartered and opposite quarters were blended. An aliquot of about 100 g was weighed and placed in a blender. Subsequently, 0.5 g of sodium carbonate and 150 ml of acetone were added and the entire mixture was blended for 3 min. The mixture was filtered and the filtrate was transferred into a separatory funnel. The extract was partitioned with 100 ml of *n*-hexane and the acetone layer was removed. The acetone layer was saturated with 20 g of NaCl and the solution was stirred for about 40 min. The saturated acetone layer was transferred to a separatory funnel, where the insecticide was extracted with 200 ml of chloroform and with two additional 100-ml portions of a mixed solution of chloroform and acetone (9:1, v/v). The three solutions were combined and dried with 50 g of anhydrous sodium sulfate. The combined solvents were then evaporated to an oily residue in a rotary vacuum evaporator at a water bath temperature of 55°. The extract was diluted to a known volume with 2-methoxyethanol. To obtain the recovery yields for this procedure, tomatoes of the control plot were used and *O,S*-dimethyl phosphoroamidothioate of 99.7% purity was added to a 100-g aliquot of macerated tomatoes. The entire analytical procedure as previously described was applied on this mixture as well as on untreated tomatoes to check for possible interferences.

**Gas Chromatographic Analysis.** The gas-liquid chromatographic analyses were performed on a Varian Model 2100 chromatograph equipped with a flame photometric detector (FPD). The FPD detector was operated in conjunction with a bypass valve, which was installed at the column exit so as to prevent the extinguishing of the flame due

**Table I. Recovery of *O,S*-Dimethyl Phosphoroamidothioate Added to Tomatoes**

| Added, ppm | Found, ppm[a] | | | % recovery |
|---|---|---|---|---|
| 0.038 | 0.031 | 0.025 | (0.028) | 73 |
| 0.19 | 0.16 | 0.12 | (0.14) | 73 |
| 0.48 | 0.39 | 0.33 | (0.36) | 75 |
| 1.00 | 0.75 | 0.69 | (0.72) | 72 |
| | | | Average | 73.2 |

[a]Values in parentheses indicate the average of replicate samples.