

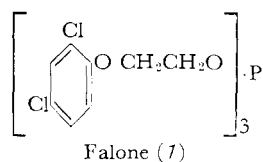
# Microdetermination of Falone Residues in Food Crops

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The work was done to satisfy government requirements for a chemical test to determine residual Falone (tris-2,4-dichlorophenoxyethyl phosphite) in treated crops. The method developed uses a new colorimetric reaction sufficiently sensitive and specific to measure 0.03 p.p.m. or more of Falone in asparagus, corn meal, cranberries, peanuts, white potatoes, and strawberries, or to determine 1 to 10  $\mu\text{g}$ . of several other dichlorophenoxy and naphthoxy compounds. The reaction depends on the ability of the intense purple color of nitrated dimethylnaphthidine in concentrated sulfuric acid to lessen in intensity in proportion to the micro quantity of the 2,4-dichlorophenoxy compound present.

**F**ALONE (tris - 2,4 - dichlorophenoxyethyl phosphite, often referred to as 3Y9) is an effective pre- or postemergence herbicide on a number of field crops (3), including peanuts (3) and white potatoes (3), on which Falone has USDA registration for use (2), strawberries (3), sweet potatoes (3), nursery turf (specifically crabgrass control) (3), corn (3), and asparagus (3). Falone has also shown considerable merit in maintaining weed control in "summer fallow" in western areas (3).



Since organic phosphite pesticides are systemic, a sensitive method was required to determine Falone residues in the various crops. A method sensitive to 0.10 p.p.m. was desirable.

Initial work using micro quantities showed that Falone readily hydrolyzes in refluxing aqueous potassium carbonate to liberate 3 molecules of 2,4-dichlorophenoxyethanol (DCPE) which will steam-distill. Since DCPE could be easily isolated by distillation, a colorimetric test was sought to detect micro quantities of DCPE. Examination of the literature revealed no colorimetric test having sufficient sensitivity and specificity to determine 0.10 p.p.m. of DCPE in the given crops.

During the search subsequently undertaken, the following colorimetric test was discovered, which proved to be extremely sensitive and had good specificity for DCPE in the presence of naturally occurring plant interferences.

## Experimental

Dimethylnaphthidine (I) dissolved in concentrated sulfuric acid containing a

small amount of potassium nitrate reacts immediately to form an intense stable purple coloration (II). If 1 to 5  $\mu\text{g}$ . of DCPE (III) is also present, it is nitrated to IV, thereby increasing the reactivity of the chlorine atoms. IV then reacts with II, causing the intensity of the purple color to decrease in proportion to the concentration of DCPE in the ratio of 0.2 absorbance unit per microgram of DCPE (Figure 1).

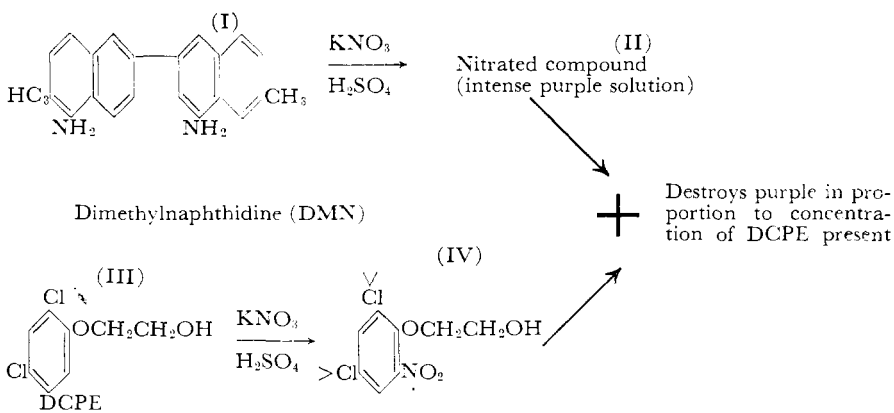


Figure 2 shows the spectrum of a reagent blank analysis (A) and a 5- $\mu\text{g}$ . DCPE standard analysis (B). The intense purple color exhibited by the reagent blank analysis at its 550-m $\mu$  absorption peak is almost destroyed when 5  $\mu\text{g}$ . of DCPE are present, as seen in curve B.

The dimethylnaphthidine color reaction described above is as intense and reactive with other dichlorophenoxy and naphthoxy - containing compounds as with DCPE. Although the rate of decrease in color intensity is somewhat less with these compounds than with DCPE, varying with the individual chemical, 5  $\mu\text{g}$ . of any one compound are easily detectable and all appear to follow Beer's law in the 1- to 10- $\mu\text{g}$ . range.

Standard curves are prepared as follows:

Appropriately graduated micro quantities of the chemical in acetone solution (10  $\mu\text{g}$ . per 0.10 ml.) are charged to 5-ml. volumetric flasks from a 0.25-ml. hypodermic syringe together with a reagent blank analysis. The acetone is evaporated under a draft of air. 3 ml. of concentrated sulfuric acid are added, and the analyses are completed as described under Color Formation.

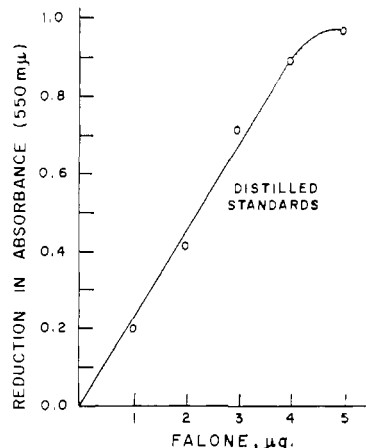


Figure 1. Falone standard color curve

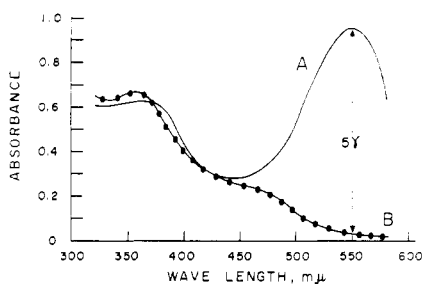


Figure 2. Absorption spectrum

- A. Reagent blank analysis  
B. 5- $\mu$ g. Falone standard analysis

The dichlorophenoxy-containing compounds, which have been found to decolorize the purple solution quantitatively, are (listed in the order of decreasing rate of reactivity):

1. 2,4-Dichlorophenoxyethanol (DCPE)
2. Tris - 2,4 - dichlorophenoxyethyl phosphite (Falone)
3. 2,6-Dichloro-4-nitrophenol
4. 2,4 - Dichlorophenoxyethyl sulfate (Crag)
5. 2,4 - Dichlorophenoxyacetic acid (2,4-D)
6. 2,4-Dichlorophenol
7. 2,4-Dichloronaphthol
8. Monochlorophenoxyethanol, gives a color destruction equivalent to  $\frac{1}{2}$  that of the dichloro compound, 1.

Phenoxyethanol and 2,4-dichlorobenzoic acid give no reaction.

The DMN color test indicates that dichlorophenoxy and naphthoxy groups exhibit a high level of reactivity, monochlorophenoxy groups reduce reactivity, while the unchlorinated phenoxy group is completely unreactive, as is also the chlorinated phenyl group which has no phenolic oxygen group. The above findings verify Equation 1, which shows that the reaction which takes place is activation of the chlorine atoms on the dichlorophenoxy group (III) by nitration (IV) and subsequent reaction with the nitrated dimethylnaphthidine (II).

**Principle.** Micro quantities of Falone hydrolyze in refluxing aqueous potassium carbonate, liberating 3 molecules of DCPE which steam distill. Carbon tetrachloride extracts the DCPE from the distillate. Concentrated sulfuric acid extracts the DCPE from the carbon tetrachloride. Addition of nitrate and dimethylnaphthidine reagents to the sulfuric acid extract then produces the color reactions described.

**Specificity.** When this procedure is employed, practically no naturally occurring substances from the crops analyzed remain at the color formation step to interfere. Hydrolysis and distillation from aqueous potassium carbonate permit distillation of only the hydrolysis product of Falone, DCPE,

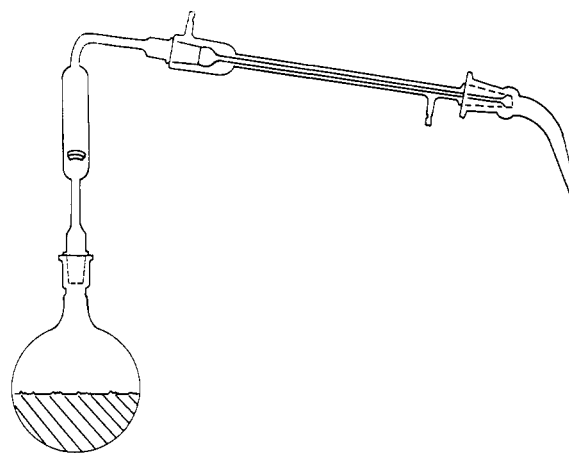


Figure 3. Falone distillation apparatus

and leave behind any 2,4-D, 2,4-dichlorophenol, or the sodium salt of 2,4-dichlorophenoxyethyl sulfate, which would produce a similar color reaction with the reagents as the distillable hydrolysis product, DCPE.

#### Reagents

**Falone Standard Solution.** Prepare an acetone or ether solution of 50  $\mu$ g. of Falone per ml.

**Dimethylnaphthidine (DMN) Reagent.** Place 0.010 gram of 3,3-dimethylnaphthidine and 30 ml. of concentrated sulfuric acid in a clean, dry, 30-ml. dropping bottle. Seal with the ground-glass pipet and swirl occasionally for an hour to dissolve the dimethylnaphthidine completely. Prepare fresh daily one hour before use.

**Nitrate Reagent.** Add 1.0 gram of potassium nitrate and 30 ml. of concentrated sulfuric acid to a clean, dry, 30-ml. dropping bottle. Swirl occasionally to dissolve completely and seal with the ground-glass pipet. This reagent is stable for a week.

**Acetonitrile,** redistilled, 81° to 84° C. fraction.

**Nitromethane,** practical grade.

#### Equipment and Materials

Use no lubricant on any ground-glass stopper, joint, or stopcock of any equipment used.

**Distillation Apparatus** (Figure 3). Heating mantle, hemispherical type, 1000-ml. capacity.

Flask, round-bottomed, short-necked, 24/40  $\text{F}$  joint, 1000-ml.

Kjeldahl connecting bulb, Iowa State type, fitted with 24/40 inner  $\text{F}$  joints on both ends.

West-type condenser, drop-tip 24/40  $\text{F}$  joints, 300-ml., jacket length.

Bent adapter, 24/40  $\text{F}$  joint.

Soxhlet extraction apparatus, Kimax (500-ml. capacity boiling flask).

Bottle, dropping with ground-in pipet, 30-ml. capacity.

Hypodermic syringe, with needle, 0.25-ml. capacity, 0.01-ml. graduations.

Centrifuge tubes, conical shape, 50-

and 100-ml. graduated, with glass stoppers.

Lapping and grinding compound, grease mixed, Grit No. 600 (extremely fine), Clover Grade 4-A.

#### Procedure

Because of procedural differences in the analysis of the various crops, a complete analytical procedure for all crops is described, which is divided into sections. Those sections necessary for the analysis of a specific crop are given with the crop in the following list.

**Corn Ears and Whole Peanuts.** Soxhlet extraction, nitromethane partition (alternate for whole peanuts, acetonitrile partition), distillation, cleanup of distillate, extraction of distillate, color formation, and calculation.

**Asparagus, Cranberries, Potatoes, and Strawberries.** Add a 50-gram sample, 175 ml. of 30% (by weight) potassium carbonate solution, 1 drop of Antifoam A, and Carborundum chips to a 1-liter round-bottomed flask. Proceed with the analytical sections: distillation, cleanup of distillate, extraction of distillate, color formation, and calculation.

#### Analytical

**Soxhlet Extraction.** To a 43  $\times$  123 mm. Whatman extraction thimble add 5 grams of anhydrous sodium sulfate followed by 50 grams of sample, and cover with another 5 grams of anhydrous sodium sulfate. Soxhlet-extract the thimble over 350 ml. of petroleum ether (30° to 60° C.) for 2 hours at a reflux rate of one siphoning per 3 minutes.

**Nitromethane Partition.** Add the petroleum ether extract to a dry, 500-ml. separatory funnel containing 40 ml. of nitromethane (practical grade). Stopper, shake vigorously for 30 seconds, and allow 2 minutes for layer separation.

Draw off the lower (nitromethane) layer into a 1-liter round-bottomed flask. Add 200 ml. of distilled water, 1 drop of Antifoam A, and Carborundum chips.

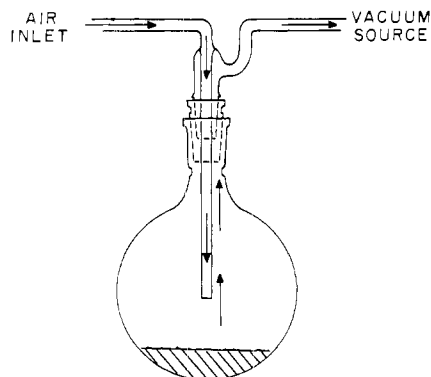


Figure 4. Acetonitrile evaporation apparatus

Seat the flask in a 1-liter heating mantle and attach to the distillation apparatus (Figure 3). With the Powerstat set at 90 units, distill, collecting nitromethane and water (immiscible layers) in a 100-ml. graduated centrifuge tube until 40 ml. of water have been collected. Discard distillate. Remove and cool the distilling flask to room temperature, add 65 grams of granular potassium carbonate, and swirl to dissolve.

**Acetonitrile Partition.** Add the petroleum ether extract to a dry, 500-ml. separatory funnel and successively extract with 40, 20, 10, and 10 ml. of acetonitrile. Filter the recovered acetonitrile through Whatman No. 4 filter paper into a 1-liter round-bottomed flask. Set the flask in a heating mantle (Powerstat at 55 units). Insert a modified gas scrubbing apparatus fitted with a 24/40 joint (Figure 4) into the neck of the flask and attach to a vacuum source. (The draft of air thus produced in the flask evaporates the acetonitrile very rapidly at or below room temperature.) Adjust the air flow in the flask to avoid excessive spattering of the acetonitrile. When all but 5 ml. of the acetonitrile have been evaporated, remove the evaporation apparatus from the mantle and with gentle swirling allow the remaining acetonitrile to evaporate without external heating. A small oil film remains, which prevents evaporation of DCPE. When evaporation of the acetonitrile is complete, remove the modified gas scrubbing apparatus from the neck of the flask and add 175 ml. of 30% (by weight) potassium carbonate solution, 1 drop of Antifoam A, and Carborundum chips.

**Distillation.** Place the flask in a 1-liter heating mantle and attach to the distillation apparatus (Figure 3) which is steamed to clean prior to each use. Set the Powerstat at 85 units and with condenser water flow rapid, collect 100 ml. of distillate.

**Cleanup of Distillate.** FOR PEANUTS USING NITROMETHANE PARTITION, AND POTATOES. No cleanup is used: Proceed with extraction of distillate.

FOR PEANUTS USING ACETONITRILE PARTITION. Collect the distillate in a marked 250-ml. Erlenmeyer flask, add 30 ml. of distilled water, and volatilize

30 ml. by boiling to remove volatile interferences. Cool.

FOR ASPARAGUS, CRANBERRIES, AND STRAWBERRIES. Acidify the distillate with 20 drops of concentrated sulfuric acid, extract with 30 ml. of nitromethane in a 250-ml. separatory funnel, and draw off the lower nitromethane layer to a 1-liter round-bottomed flask containing 130 ml. of distilled water and Carborundum chips. Attach the flask to the distillation apparatus (Figure 3) and distill and discard nitromethane plus 30 ml. of water. Cool the residue in the distilling flask to 30° C. for extraction.

**Extraction of Distillate.** After cleanup acidify the distillate with 20 drops of concentrated sulfuric acid and extract with 40 ml. of carbon tetrachloride in a 250-ml. separatory funnel. Filter the lower carbon tetrachloride layer through Whatman No. 4 filter paper into a clean, dry 50-ml. conical centrifuge tube fitted with a ground-glass stopper. Add 2 grams of anhydrous sodium sulfate, stopper the centrifuge tube, shake 30 seconds, and allow to stand 10 minutes. Decant the dried carbon tetrachloride and filter through Whatman No. 4 filter paper into a dry 60-ml. separatory funnel. Add 3 ml. of concentrated sulfuric acid from a 25-ml. buret, stopper, and shake vigorously for 30 seconds. Swirl gently and let stand 20 minutes for layer separation. Draw off the lower concentrated sulfuric acid layer into a dry 5-ml. volumetric flask.

**Color Formation.** First prepare a reagent blank to determine the intensity of purple available from a given amount of freshly prepared DMN reagent. Add 3 ml. of concentrated sulfuric acid to a dry 5-ml. volumetric flask followed by 2 drops of nitrate and 10 drops of DMN reagents. Horizontally agitate the volumetric flask to mix the contents thoroughly and allow to stand 30 minutes to simulate an actual analysis. Then measure the reagent blank at 550 m $\mu$  in a 1-cm. cell, using water as the reference in a Beckman Model DU spectrophotometer. Use the absorbance value thus obtained to estimate the number of drops needed to produce an initial optimum purple intensity of 1.6 to 1.7 absorbance units in ensuing sample analyses.

Add the color reagents to a complete set of samples to ensure similar reagent activity. Carry an untreated, an untreated fortified with 0.1 p.p.m. of

Table I. Recoveries of Falone Added to Untreated Crops

Crop	Added, P.P.M.	No. of Detns.	Recovered, P.P.M. <sup>a</sup>	Recovery, %	Av. Recovery, %
Asparagus	0.10	2	0.07, 0.08	71, 78	75
Corn ears	0.10	22	0.06-0.10	63-100	74
Cranberries	0.10	2	0.07, 0.07	66, 72	69
Peanuts	0.10	23	0.05-0.09	51-92	71
Potatoes	0.10	7	0.07-0.10	68-97	87
Strawberries	0.10	7	0.06-0.10	60-100	83

<sup>a</sup> Corrected for interference from untreated samples.

Falone, and duplicate analyses of one or two accompanying treated samples together with a reagent blank analysis through the prescribed analytical procedure to obtain the concentrated sulfuric extracts in the 5-ml. volumetric flasks. Carefully add 2 drops of nitrate reagent to each volumetric flask, followed by the number of drops of DMN reagent necessary (as estimated above) to provide a purple intensity of 1.6 to 1.7 absorbance units. (The drops of the reagent added should fall directly to the bottom of the 5-ml. flasks without touching the neck.) Agitate the volumetric flasks to mix the contents and let stand 30 minutes. Transfer the color solutions to an equal number of clean, dry 1-cm. cells and measure together at 550 m $\mu$ , using water as the reference.

**Calculation.** Determine the amount of Falone in the sample by referring the net absorbance (the difference between the absorbance of an untreated sample and a treated or fortified sample) to a standard curve—i.e., Figure 1—prepared by processing aliquots of Falone standard solution as described below.

To 1-liter round-bottomed flasks containing 175 ml. of 30% potassium carbonate solution, add appropriately graduated micro quantities of Falone—i.e., 1, 2, 3, 4, and 5  $\mu$ g.—as an acetone solution of technical Falone. (The Falone content of technical material is assumed to be equivalent to the chlorine content as determined by a total chlorine analysis.) Attach the 1-liter flask to the distillation apparatus (Figure 3) and take 100 ml. of distillate. Then complete the analyses as described in the procedure, beginning with the extraction of distillate.

## Discussion

**Effects of Moisture on Color Reaction.** SAFEGUARDS. Traces of water in the concentrated sulfuric acid extracts or in the color reagents will inhibit the color reaction. Extreme caution must be observed to provide dry 60-ml. separatory funnels, dry 30-ml. dropping bottles for reagents, dry 50-ml. centrifuge tubes, dry 5-ml. volumetric flasks, dry 1-cm. cells for absorbance measurements, and a dry 25-ml. buret for dispensing the 3-ml. aliquots of sulfuric acid for the extraction of the carbon tetrachloride.

The above glassware is cleaned with warm water, followed by an acetone rinse, and dried in an oven at 100° C. When Falone analysis is begun, a new 10-pound bottle of concentrated sulfuric acid is fitted with a screw-on type of plastic adapter to hold an automatic 100-ml. graduated dispensing buret. All sulfuric acid needed for reagents and analyses is drawn, as needed, from this system to minimize contamination of the acid by air moisture.

No lubricant is used on any stopcocks or other ground-glass joints, because of possible contamination of the organic solvents and concentrated sulfuric acid which come in contact with the joints. To ensure that the unlubricated stopcocks of the separatory funnels are leak-proof and easy turning, the stopcocks are ground into their joints with grease-mixed, valve grinding compound of very fine grade. The compound is removed with successive rinses of hexane, acetone, and water.

**Processing of Samples.** Dry crops—i.e., husked corn ears and whole peanuts—are ground to a meal in a Wiley No. 2 mill. Watery crops—i.e., asparagus, cranberries, potatoes, and strawberries—are ground to a soup in a blender, and analyzed immediately, or frozen in sealed glass jars and stored. Asparagus and potatoes exhibit less interference if they are analyzed immediately after processing.

**Preparation of Falone - Fortified Samples.** A sample of 0.10 ml. (5 µg.) of the Falone standard solution (see Reagents) dispensed from a 0.25-ml. hypodermic syringe is charged to a 50-gram sample. With dry crops the Falone is charged to the sample in the Soxhlet thimble prior to extraction. With watery crops the Falone is added to the sample in the 1-liter flask prior to distillation.

**Alternative Cleanup in Strawberry Analysis.** Analyzed as for potatoes, the carbon tetrachloride extract of the distillate is scrubbed with 30 ml. of 50% (v./v.) sulfuric acid and rinsed with 100 ml. of distilled water before continuing. Similar recovery data are obtained with either cleanup.

**Analysis.** For rapidity of testing, several distillation apparatus are operated simultaneously. When a set of samples—i.e., an untreated, untreated

fortified with 0.10 p.p.m. of Falone, duplicate analyses of one or two accompanying treated samples, and a blank analysis—has been distilled, and the distillate has been cleaned up, extracted with carbon tetrachloride, and dried over anhydrous sodium sulfate, the set is transferred to a rack of dry 60-ml. separatory funnel and extracted with 3 ml. of concentrated sulfuric acid; 20 minutes are allowed for phase separation. The lower sulfuric acid phase of all the crop analyses should be light yellow, of equal intensity, and possibly have a very slight turbidity. (This turbidity disappears during the color reaction.) If one analysis appears to be noticeably more intensely yellow or to have a greater turbidity than the others, a previous part of that analysis was improperly carried out and the analysis is discarded. Next, the lower sulfuric acid layer is drawn off very slowly into the 5-ml. volumetric flask to ensure that none of the carbon tetrachloride layer comes with it. A drop of carbon tetrachloride inadvertently passed on with the sulfuric acid produces a turbidity which remains, making the absorbance measurement unreliable. All colored solutions must be crystal clear for reliable absorbance measurements.

**Standard Curve.** A standard curve should be prepared at each laboratory initially and whenever a new 10-pound bottle of concentrated sulfuric acid or Falone standard solution is used.

**SULFURIC ACID.** The presence of relatively strong concentrations of nitrate in some analytical grade sulfuric acid is indicated by a rapid development of purple color upon mixing DMN and sulfuric acid in reagent preparation (1 minute or less), whereas low nitrate is indicated by a slowly developing weak purple. In the extraction of distillate step, sulfuric acid-containing nitrate extracts some interference from the carbon tetrachloride (possibly due to the nitration of natural components causing them to partition in to the acid phase). Nitrate in bottle acid is removed by boiling 300-ml. portions in clean, dry Kjeldahl flasks on a digestion rack for 0.5 hour, and storing the cooled acid as described above. The use of boiled sulfuric acid for analysis requires eight drops of nitrate reagent in the color reaction step in place of the two drops used with the unboiled acid. Although

boiled sulfuric acid is not mandatory for general analysis, it does afford the following advantages. The standard curve is 10% more intense, yet similar in shape to a curve obtained with unboiled acid. The deviation from the mean of a series of recoveries is reduced and duplicate analyses on treated samples are more reproducible.

**Nitromethane as a Partitioning Solvent.** While acetonitrile satisfactorily partitions Falone from petroleum ether extracts of peanuts (Table I), it extracts much interference from the petroleum ether extract of corn ears. In the search for a better partitioning solvent, nitromethane appeared to be extremely selective in partitioning Falone from petroleum ether extracts of corn meal. A single extraction with nitromethane removes essentially all Falone from the petroleum ether, yet extracts no fats or waxes, whereas four successive extractions with acetonitrile extract less Falone and there is some interference. Initial analyses of peanuts using nitromethane for partitioning show it is superior to acetonitrile in its ability to extract Falone, to extract no fats or waxes, and to reduce the time per analysis.

The color reaction (as indicated by fortified samples) appears to be completed about 5 minutes faster in asparagus and strawberry analyses than in standard analysis and about 5 minutes slower in peanuts and corn ear analyses. Plant constituents apparently aid or reduce the ability of the nitrate reagent to produce the desired reaction.

#### Acknowledgment

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