Colorimetric Method for Estimation of Guthion Residues in Cottonseeds and Cottonseed Oil

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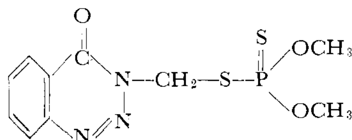
A colorimetric method is presented for the quantitative estimation of residues on cottonseeds containing Guthion. This insecticide has been used to control boll weevil and other cotton insects. Crushed cottonseeds are extracted with chloroform, and Guthion is separated from the extract chromatographically with activated alumina as the adsorbent and pentane and acetonitrile as solvents. Guthion is hydrolyzed with acid, and the formaldehyde formed is distilled and determined colorimetrically with chromotropic acid reagent at 570 mμ.

The recent interest in the use of Guthion for the control of boll weevil (*6, 12, 16*) and a number of other cotton insects (*6–8, 15, 17, 18*) has resulted in the need for a sensitive method for the estimation of residues of this toxicant present in cottonseed products, especially the oil and meal, which may be used as food for warm-blooded animals.

Guthion [*O,O*-dimethyl-*S*-(4-oxo-3H-1,2,3 - benzotriazine - 3 - methyl) - phosphorodithioate, or Bayer 17147] has the following formula:



It is practically insoluble in water (1 to 30,000), but is soluble in many organic solvents (*4*). Its volatility is very low, and its vapor pressure is lower than that of parathion.

Wollenberg and Schrader (*19, 20*) have described a colorimetric test for Guthion based on opening the triazine ring and coupling with phenyl-1-naphthylamine in acetic acid to give a blue-violet color. No details of the application of this procedure to residue analysis have been given. Recently a method for Guthion residues on cottonseeds has been developed (*5*) based on its hydrolysis to anthranilic acid, followed by diazotization and coupling with naphthylethylenediamine.

The method described in this paper is based on the observation that Guthion can be easily hydrolyzed by acid at a moderately high temperature to give formaldehyde, from the methylene group, as one of the products. The procedure consists in extraction of ground cottonseeds with chloroform in a Soxhlet extractor, evaporation of the solvent, separation of Guthion residues from the extract by special two-solvent chromatography, acid hydrolysis of the residue, and distillation of the formaldehyde. The formaldehyde is determined colorimetrically with chromotropic acid reagent at 570 mμ.

The analysis for Guthion residues in cottonseeds is complicated by the large content of oil (approximately 20%). Oily or fatty materials present problems in most residue analyses, particularly with nonvolatile oil-soluble phosphates, as the separation or cleanup procedures must be mild unless total phosphate is being determined.

A number of techniques have been used for the separation of insecticides from oil or fat. Jones and Riddick (*13*) isolated several insecticides from butterfat by repeated partitions of the extract between *n*-hexane and acetonitrile. Burchfield and Storrs (*3*) used dimethylformamide for the same purpose. Similarly, Erwin *et al.* (*10*) employed reverse-phase partition chromatography in their isolation of pesticides from extracts of animal tissues. They used wax-coated alumina as the adsorbent and acetonitrile-water as the eluent. In olive or corn oil samples a preliminary extraction with an equal volume of acetonitrile separated the insecticides without serious loss. Kolbezen and his associates, in their method for Chlorthion [*O*-(3-chloro-4-nitrophenyl)-*O,O*-dimethyl phosphorothioate] in cottonseeds (*14*), extracted Chlorthion from a pentane solution with acetonitrile followed by chromatography on a short column of activated alumina. The authors tried these methods for Guthion residues in cottonseeds, but found that the cleanup was not sufficiently good.

Although in the absence of oil Guthion could be readily extracted from pentane by acetonitrile, the presence of 20 to 40 grams of cottonseed oil in pentane affected the partition ratio so adversely that even with six or seven extractions inadequate recovery of Guthion was obtained. Therefore a special chromatographic technique was devised, in which cleanup by chromatography and partitioning into acetonitrile are both accomplished on an alumina column to secure a good separation of the Guthion from cottonseed oil with adequate elimination of interferences.

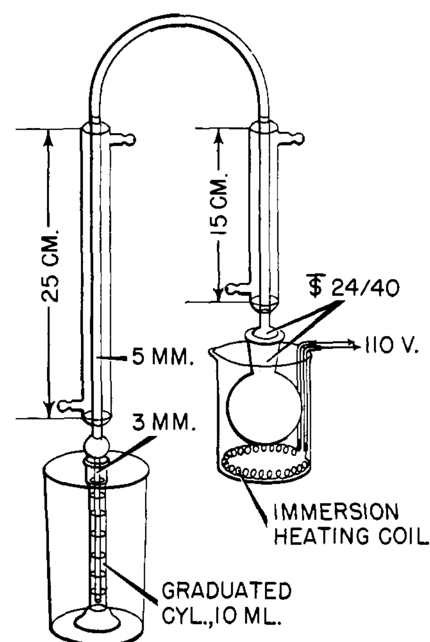


Figure 1. Steam distillation unit

Apparatus

Photoelectric colorimeter, Beckman Model B, or equivalent.

Colorimetric cuvettes, Corex D glass, matched, 1-cm. light path.

Chromatographic tubes with a cold-water jacket (20 × 400 mm.).

Steam distillation unit (all glass) (Figure 1).

Oil bath, Fisher bath wax, or equivalent, flash point 325° C.

Borosilicate glass test tubes, 15 × 150 mm., with ground-glass stoppers. Scratch a mark on the glass at 5-ml. volume.

Soxhlet extractor unit, large enough to extract 200 grams of crushed cottonseeds. (One with an extraction tube

having a 70-mm. inside diameter and a 71/60 ₸ joint between tube and condenser was satisfactory.)

Reagents

Chloroform, technical grade. Wash the solvent four times with successive equal volumes of water. Dry over anhydrous calcium chloride and distill.

Celite 521 (Johns-Manville Co.).

Colorimetric pentane (Phillips Petroleum Co.).

Acetonitrile, boiling point 80–81° C. (Union Carbide Chemicals Co.), redistilled.

Alumina. To 1 pound of adsorption alumina, 80 to 200 mesh (Merck Chemical Co.), in a large flask add 800 ml. of distilled water and 5 ml. of concentrated hydrochloric acid and mix thoroughly. Let stand at room temperature for at least 2 hours, wash by decantation four times with water, filter through a Büchner funnel, and continue washing until the filtrate is very faintly acid. Wash the alumina with two 400-ml. portions of ethyl alcohol, dry in an oven at 140° C. overnight, and store in a tightly closed container.

Chromotropic acid reagent. With the aid of heat dissolve 16 grams of chromotropic acid (sodium salt of 4,5-dihydroxy - 2,7 - naphthalenedisulfonic acid, practical) in concentrated sulfuric acid (C.P. grade), cool to room temperature, and dilute to 1 liter. Store in a brown bottle or in a bottle wrapped in brown kraft paper, and dispense with an automatic buret. Make fresh reagent every 2 weeks.

Guthion, technical grade, is recrystallized from pentane and carbon tetrachloride (melting point 73–74° C.).

Procedure

Weigh accurately 50 mg. of Guthion, transfer to a 500-ml. volumetric flask, and make up to the mark with chloroform. After mixing, pipet 50 ml. of the solution into a 100-ml. volumetric flask, make up to the mark with chloroform, and mix well. Pipet aliquots of the second solution into a series of 100-ml. round-bottomed flasks so that these flasks will contain 0, 100, 200, 300, or 400 micrograms of this ester. Add a glass bead to each flask, and carefully evaporate the solvent just to dryness on the steam bath. Remove the last trace of chloroform at room temperature with a slow jet of air. To each flask add 40 grams of crude cottonseed oil and 40 ml. of pentane, and mix well.

Prepare the chromatographic column by placing 60 grams of activated alumina in the water-cooled chromatographic tube, packing the adsorbent down by tapping the sides of the tube. Prewash by percolation with 100 ml. of pentane. When the solvent level reaches the top of the alumina column, introduce the oil sample in four or five small portions until all the oil solution has been added. Measure 125 ml. of pentane in a graduated cylinder, and use small portions to rinse the flask, adding each rinse to the column until all the pentane is used. With the same cylinder measure 125 ml. of acetonitrile. Use 25 ml. to rinse the flask carefully. When the last portion of pentane reaches the top of the column, add the acetonitrile rinse and let it run down to the top of the column. Discard all the pentane collected from the column thus far. Add the remainder of the acetonitrile, and from this point on collect all the pentane (a few milliliters at most) and the acetonitrile percolated through the column in a Kuderna-Danish concentrator (*11*). Add one or two glass beads to the 100-ml. round-bottomed flask that was earlier used for the same oil sample, and attach it to the evaporative concentrator. Evaporate off the solvents carefully on the steam bath. Remove flask from the steam bath and evaporate last trace of acetonitrile with a slow jet of air.

For the acid hydrolysis and distillation, pipet 1 ml. of water into a 10-ml. graduated cylinder, which is kept in an ice bath, as the receiver. Add 15 ml. of concentrated hydrochloric acid to the round-bottomed flask containing the Guthion residue, and quickly attach it to the distillation unit (Figure 1). The distillation unit should be set up in a hood, as some hydrochloric acid fumes are evolved. Turn water on in the 15-cm. condenser and reflux the Guthion residue in an oil bath for 20 minutes. Then turn off and empty the water in the 15-cm. condenser, but turn water on in the 25-cm. condenser and distill at a bath temperature of about 130° to 160° C., until the total volume in the graduated cylinder is exactly 10.0 ml. Keep the end of the delivery tube under the surface of the distillate until near the end of the distillation. Then lower the ice bath and the cylinder so that the tip of the delivery tube is just above the surface of the distillate until the distillation is completed.

To develop the color, place 2 ml. of the distillate in a borosilicate glass test tube, and cool in an ice bath. Introduce slowly into the tube 3 ml. of the chromotropic acid reagent from the automatic buret (in a hood). Mix by gentle swirling and tapping. Stopper loosely with a glass stopper, and place the tube in a boiling water bath. After 60 minutes remove the tube from the bath and cool it at once in a beaker of cold water. Dilute the contents of the tube to the 5-ml. mark with hydrochloric acid and mix well. Fill a cuvette with the solution, cover, and measure in the photometer at a wave length of 570 mμ against the reagent blank solution, prepared by mixing 2 ml. of hydrochloric acid and 3 ml. of chromotropic acid reagent and heat in boiling water for 60 minutes.

Prepare the standard curve by plotting the absorbance readings against micrograms of the insecticide present. A standard curve, *E*, is shown in Figure 2.

Table I. Recovery of Guthion from Crushed Cottonseeds

Added		Recovered		
γ	P.p.m.	γ	P.p.m.	%
50	0.25	47	0.24	94.0
100	0.50	94	0.47	94.0
200	1.00	192	0.96	96.0
400	2.00	390	1.95	97.5
800	4.00	792	3.96	99.0

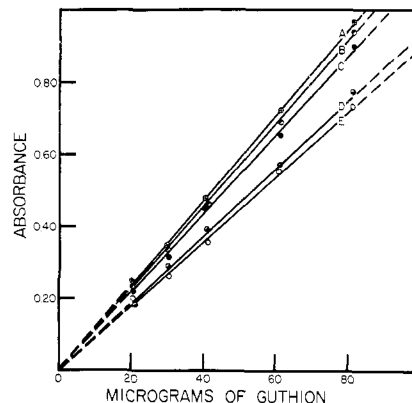


Figure 2. Losses of Guthion at various stages in the analytical procedure

A. Evaporation of aliquots of standard solution in chloroform
B. Addition and evaporation of 100 ml. of acetonitrile
C. Distillation
D. Chromatography
E. Addition of 40 grams of crude cottonseed oil to sample

Analysis of Cottonseed Samples

Grind the sample of whole cottonseeds in a Wiley mill, and extract a weighed amount of the crushed sample in a Soxhlet extractor with chloroform for 16 hours. Chill the extract in an ice water bath and filter the solution with vacuum through a thin layer of Celite 521 in a Büchner funnel. Remove or recover the solvent from the extract on the steam bath. After complete removal of the solvent, proceed with the analysis as described in the preparation of the standard curve, starting by dissolving the oil in an equal volume of pentane, followed by chromatographic separation of the Guthion residue from the oil.

Recovery Tests with Method

The recovery of Guthion, added to crushed cottonseeds, was determined by adding known amounts in chloroform to a series of 200-gram samples and then extracting with chloroform in a Soxhlet extractor and analyzing. Results are based on curve *E*, Figure 2 (Table I).

The analytical procedure was checked

to determine the losses which occur at various steps (Figure 2). Curve A, Figure 2 shows the development of color after evaporation of a series of aliquots of the standard solution. Curve *B* is obtained after evaporation of 100 ml. of acetonitrile added to each of a second series of aliquots. Curve *C* results from the same steps as curve *B*, but includes the additional steps of refluxing with acid and distillation. The points for curve *D* were obtained after the fourth series of aliquots was chromatographed and the acetonitrile was evaporated, followed by refluxing with acid and by distillation. Curve *E* results from the addition of the fifth series of aliquots to 40-gram samples of crude cottonseed oil and running the samples through the complete analytical procedure. It can be seen from Figure 2 that the over-all recovery (curve *E* as compared to curve *A*) was 70 to 75%. The recovery was reasonably constant, and residues greater than 0.25 p.p.m. could be satisfactorily determined. A series of samples of cottonseed oil containing 5 p.p.m. of Guthion were analyzed and the following results were obtained: 4.8, 5.0, 4.6, 4.6, 5.0, and 4.8 p.p.m. based on curve *E* (Figure 2) or 3.9, 4.0, 3.6, 3.6, 4.0, and 3.8 p.p.m. based on curve *A*. The averages were 4.8 p.p.m. (curve *E*) or 3.8 p.p.m. (curve *A*) with standard deviations of 0.16 or 0.17, respectively. Based on curve *A* this is a recovery of approximately 75%, whereas based on curve *E* the recovery is over 94%.

Discussion

Chromatography. The use of a chromatographic column in the manner described has certain features that commend it for this particular isolation problem, and the technique may prove advantageous for the isolation or cleanup of other materials. The principles involved are as follows.

Guthion is not very soluble in pentane, and therefore should be readily adsorbed on the column in the first step, which can be considered as ordinary adsorptive chromatography on alumina with pentane as a solvent. Washing the column with pentane elutes much of the oil, while the Guthion remains strongly adsorbed on the column. The amounts of pentane and adsorbent must be adjusted so that most of the oil can be eluted before the band of adsorbed insecticide reaches the bottom of the column.

The second step consists in the elution of Guthion by means of acetonitrile, which is immiscible with pentane. Ordinarily, the use of one solvent on a column followed by an immiscible solvent is not considered good chromatographic practice. In partition chromatography the ratio of the amount of immobile solvent to that of adsorbent is usually kept below a certain critical limit to prevent blocking or channeling. In the present work, following the pentane wash immediately by acetonitrile caused no difficulties and was advantageous for a number of reasons.

The elution of the adsorbed oil from the column with pentane, although removing most of the oil, still leaves some lipoid material on the column. Washing of the column with more than 125 ml. of pentane leads to some loss of Guthion. The use of acetonitrile after the pentane wash readily elutes the Guthion, and possibly there is some reversed-phase partition-chromatographic action, although it is likely that most, if not all, of the pentane adhering to the adsorbent is finally displaced by the strongly polar acetonitrile. Most of the pigments and interferences are still left on the column after the acetonitrile elution. The small amount of displaced pentane containing some lipoid materials in the eluent does not seem to interfere in the final color development.

The cleanup procedure with suitable modifications in type and amounts of adsorbent and solvents can probably be used for other insecticide residues or other difficultly isolatable compounds. Even for Guthion analyses, the analyst may have to adjust the relative quantities of solvent and adsorbent used, because the adsorbent may vary in activity from one batch to another.

Blanks and Controls. It is important to run control analyses of samples of cottonseeds or oil containing no Guthion. The use of a lower concentration of reagent than that suggested by Beroza (*1*) and Bricker and Johnson (*2*) was found to reduce blank readings by about one half, with a decrease of only 10% in the intensity of color given by Guthion or formaldehyde.

Hydrolysis of Guthion. For hydrolysis of Guthion and distillation of the formaldehyde formed, a number of strong acids and various dilutions of them were studied. Strong phosphoric and sulfuric acids are not suitable in this work, because they give high readings in the blank samples and as a result the determination of small quantities of Guthion becomes troublesome. Concentrated hydrochloric acid was found to be better than dilute acid. With concentrated hydrochloric acid all the formaldehyde is distilled over in less than 9 ml. of distillate at a bath temperature of 130° to 160° C. The distillate is collected in an ice water bath to minimize the escape of hydrochloric acid fumes. In the distillation, the use of a stream of air to sweep the formaldehyde over and collecting it in water in the receiving tube, was tried but was inadequate.

The hydrolysis of Guthion in the distillation flask is complete and all the formaldehyde is distilled over within the first 6 ml. of distillate if 15 ml. of concentrated hydrochloric acid is used. One milliliter of water is placed in the receiver to trap any formaldehyde formed initially, and 9 ml. more is collected for the complete distillation. The last portion of the distillate washes down the delivery tube after it is raised.

Interferences. Although the analytical method has been described and recovery data have been given for Guthion itself, any degradation or metabolic products of Guthion that could yield formaldehyde would also give the test if not removed during the cleanup. The "oxygen analog of Guthion" [*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl)-phosphorothiolate, obtained by Chemagro Corp. from G. Schrader's laboratory in Germany] gives formaldehyde in the test and added known amounts can be recovered from crushed cottonseeds.

2,4-D and related herbicides and growth regulators have been found to liberate formaldehyde on being heated with acids (*9*). However, with this method, 2,4-D did not interfere.

Most of the insecticides tried—such as DDT, BHC, chlordan, aldrin, malathion, dieldrin, parathion, demeton, schradan, and Phosdrin—do not interfere. However, certain of them do liberate formaldehyde on acid hydrolysis—such as Thimet, Trithion, Nialate [*S,S*-bis(*O,O*-diethyl phosphothioate)] and Bayer 16259 (diethyl homolog of Guthion)—and these could be considered as interferences. It should be possible to use the same procedure, with suitable modifications, to determine these other formaldehyde-liberating pesticides.

Preliminary experiments on certain nonoily crops, such as peaches, have indicated that in the absence of oil the crop may be extracted with chloroform, pigments and interferences may be removed by filtration of the chilled chloroform solution through a mixture of Celite 521 and decolorizing carbon (the carbon should be washed with water and dried) in a Büchner funnel, and the analyses for Guthion residues made, omitting the chromatographic cleanup.

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INSECTICIDE RESIDUES

Colorimetric Determination of Heptachlor in Soils and Some Crops

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When heptachlor was determined in soils and some crops, extraction by tumbling, using a mixture of colorimetric pentane and acetone (4 to 1) and a subsequent cleanup with activated carbon, gave consistent results with recoveries of approximately 90%. The method is fast and allows a considerable saving of colorimetric pentane as compared with the method using a 10-gram Florex column for cleanup. Heptachlor in soils and crops was determined according to the method of Polen and Silverman.

HEPTACHLOR (1,4,5,6,7,8,8-heptachloro - 3a,4,7,7a - tetrahydro-4,7-methanoindene), after being isolated from soils or crops, was determined according to the Polen and Silverman method (*2*). It reacted with the Polen and Silverman reagent and the pink to violet color obtained was measured photometrically at 567 mμ against benzene–isopropyl alcohol (4 to 1) as a reference. Soils and various crops, treated with heptachlor in pentane, were extracted initially with colorimetric pentane in a Soxhlet extractor and passed subsequently through a 10-gram Florex column. Extremely low and erratic recoveries were encountered and reproducible results could not be obtained.

The principal loss of the heptachlor was in the chromatographic procedure. Batches of Florex obtained at different times varied considerably in their retention of both the heptachlor and interfering substances. Recoveries of known amounts of heptachlor added to Florex columns varied between 26 and 92%, with most recoveries being below 70%. When a mixture of colorimetric pentane–acetone in a 4 to 1 ratio was used, the recoveries were 11 to 15% higher than with an extraction with colorimetric pentane only. Crops or soils extracted with pentane and acetone gave an apparent heptachlor content of less than 0.1 p.p.m. Difficulties were also encountered with Soxhlet extractors, especially when soils had to be extracted. Inconsistent and sometimes low recoveries were obtained, most probably due to channeling.

The following procedure proved to be satisfactory.

Extraction Procedure

All materials under investigation were extracted by tumbling with a mixture of colorimetric pentane–acetone (4 to 1). Two-quart, wide-mouthed Mason jars were used and a 1-hour head to end tumbling was applied.

Soils. The amount of soil to be extracted depended on the amount of toxicant present. Where low residues were expected, more soil was taken. A maximum of 400 grams of soil could be extracted safely. The soil under investigation was extracted under moisture conditions similar to those prevailing in nature. With dry soil or soils which were too wet, extraction of the toxicant was incomplete. A mixture of colorimetric pentane–acetone (4 to 1) and anhydrous sodium sulfate, equal in weight to that of the soil, were added to the extraction jar. Two milliliters of solvent were used per gram of wet soil. An additional 100 grams of soil were dried for 24 hours at 50° C. to determine the dry weight of the soil.

Crops. The edible portion of the crop material was finely ground in a food chopper. Usually 100 grams were weighed out, placed on paper, and mixed with 200 grams of anhydrous sodium sulfate. During the drying time (about 0.5 hour) the crop and the sodium sulfate were mixed several times. When 100 grams of plant material were used for extraction, a mixture of 400 ml. of colorimetric pentane–acetone (4 to 1) was added to the crop–sodium sulfate mixture in the Mason jar. After tumbling, the extraction jars were placed into a refrigerator for 0.5 hour, to minimize the evaporation of pentane during filtration. After cooling, the supernatant liquid was decanted through glass wool. The recovered volume was recorded at room temperature to be used as a factor in the calculation of the results.

The acetone was removed from both soil and crop extracts by washing first with water and then with a saturated solution of sodium chloride. The extract was dried over sodium sulfate and concentrated to about 30 ml. on a 50° C. bath, using a Vigreux column.

Removal of Interfering Substances

From Soil Extracts, Including Muck Soil. Nuchar activated carbon (C 190-N, pH 6), 0.5 gram, was added to the concentrated extract. The mixture was swirled gently for 1 minute. After an additional 5 minutes, it was filtered through a 1/2-inch layer of asbestos with glass wool on top. Chromatographic columns, 7 × 3/4 inch with sintered glass disk bottoms, were used.

Slight pressure was applied when the filtration was slow. After several washings with colorimetric pentane from a wash bottle, the clear eluate was concentrated to approximately 15 ml. and then adjusted to 25 ml. in a volumetric flask. Aliquots of the whole extract, depending on the estimated amount of heptachlor present, were then used for analysis. This cleanup procedure allows a considerable saving of colorimetric pentane as compared with the method using a 10-gram Florex or Florisil column. As specific recovery tests were run with each analysis, the Nuchar used was tested each time.