PYRETHRUM ANALYSIS

Rapid Colorimetric Determination Of Total Pyrethrins by Reaction with Sulfur

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Research on pyrethrum, evaluation of pyrethrum flowers, and quality control of pyrethrin-containing insecticides have been hampered by the lack of quick, reliable, and sensitive methods for the determination of pyrethrins. A quick, simple method, capable of giving results of good reproducibility, has been developed. It permits the determination of total pyrethrins down to a concentration of about 0.04 mg. per ml., based on the measurement of a red color developed by pyrethrum extracts upon addition of sulfur solutions in alcoholic potassium hydroxide and in carbon tetrachloride. Kerosine, synergists, or DDT do not affect the results. The method has been successfully applied for the rapid determination of total pyrethrins in pyrethrum flowers and in commercial, oil-based insecticides. The method may aid in the rapid classification of pyrethrum flowers, the evaluation of flower extraction processes, and plant control of commercial insecticides.

PYRETHRINS MAY BE DETERMINED by several methods. The Seil method (*5*) and the mercury reduction method (*1*) have been extensively used, but they require an excessive time for each analysis and their reproducibility is not satisfactory. The recently developed spectrophotometric procedure (*6*) and the ethylenediamine method (*4*, *7*) are more rapid and capable of giving results of excellent reproducibility. Nevertheless, the spectrophotometric procedure requires a sample that is free of all ultraviolet-absorbing material other than the pyrethrins. As a consequence, any petroleum oil residue must be carefully eliminated and the method is not applicable to pyrethrum solutions also containing piperonyl butoxide or DDT. The ethylenediamine method overcomes some of these drawbacks, but requires relatively large amounts (250 mg.) of pyrethrins for each determination, which makes it impractical for direct analysis of commercial pyrethrum insecticides. While the present work was in progress, a colorimetric method for pyrethrin determination in paper coatings was published (*2*).

The colorimetric procedure herein described gives values for the total pyrethrin content of test samples. It is applicable to solvent-free flower extracts, as well as to kerosine solutions of pyrethrins. The generally used synergists or the insecticide DDT do not interfere with the method. The procedure is quick and simple and gives results of good reproducibility.

Feinstein (*3*) recently reported that a solution of 2-(2-aminoethylamino)-ethanol and alcoholic potassium hydroxide will give a red or violet color with pyrethrins or allethrin, if sulfur is added. The authors have found that a red to brown color is developed when solutions of sulfur in alcoholic potassium hydroxide and in carbon tetrachloride are added to extracts of Ecuadorean pyrethrum flowers. The intensity of this color, developed under controlled conditions, is proportional to the amount of pyrethrins in the sample, as determined spectrophotometrically. Beer's law is followed within a suitable range. The method is sensitive. It has been extensively used in this laboratory and has proved very useful for control analysis of the pyrethrin content of flowers and extracts.

Table I. Effect of Relative Proportion[a] of Pyrethrins I and II

Sample	Pyrethrins, Mg./Ml.[b]	Ratio[b] Pyrethrins I/Pyrethrins II	Klett-Summerson Reading, 540 Mμ
1	1.0	3.00	270
2	1.0	1.17	270
3	1.0	0.80	270
4[c]	1.0	0.56	268

[a] Measurements carried out by W. L. Johnson, U. S. Industrial Chemicals Co., whose helpful assistance is gratefully acknowledged.
[b] By AOAC method (*1*).
[c] Vacuum distillation residue.

Method

Apparatus Constant temperature water bath, maintained at 30° ± 0.5° C. Test tube basket to fit water bath. Photoelectric colorimeter, Helige-Diller Model 500, or equivalent.

Materials for Reagents Anhydrous ethyl alcohol. Potassium hydroxide, C.P. reagent. Flowers of sulfur. Carbon tetrachloride, C.P.

Procedure **Preparation of Sulfur-Potassium Hydroxide Reagent.** Mix 0.25 gram of flowers of sulfur with 1000 ml. of anhydrous ethanolic potassium hydroxide, approximately 1*N*. Let stand, at room temperature and with occasional shaking, until solution is complete (usually overnight). If

there is a faint cloud, centrifuge before using, keeping protected from air and moisture. (Hydration of the reagent will result in a heterogeneous reaction mixture at the time of the analysis and oxidation will darken the reagent.)

Preparation of Sulfur–Carbon Tetrachloride Reagent. Mix 2.50 grams of flowers of sulfur with 1000 ml. of reagent grade carbon tetrachloride. Let stand, at room temperature and with occasional shaking, until completely dissolved.

Table II. Color Developed by Some Compounds[a] Related to Pyrethrins

(1 mg. of each compound tested)

	Klett-Summerson Reading
Ethyl chrysanthemumate	25
Allethrolone	40
Pyrethrins (Congo extract)	290

[a] Data supplied by W. L. Johnson, U. S. Industrial Chemicals Co.

Choice of Filter for Colorimetric Reading. For amounts of pyrethrins between 0.2 and 1 mg. per ml. use a 560 mμ filter. For extremely small quantities of pyrethrins (0.04 to 0.2 mg.) a blue (440 mμ) filter can be used. In this latter case the colorimetric readings are about three times larger than those obtained at 560 mμ.

Preparation of Samples. Dissolve in, or dilute with, kerosine a measured amount of the sample to be tested, so that it will contain from 0.2 to 1.0 mg. of pyrethrins per ml. if a 560 mμ filter is to be used for colorimetric reading. At the same time take a solution of known pyrethrin concentration and prepare a standard containing 0.5 mg. of pyrethrins per ml.

Development and Reading of Color. Place exactly 1.0 ml. of the solution to be tested in a 24 × 150 mm. test tube and add exactly 3 ml. of the sulfur–carbon tetrachloride reagent, followed by 3 ml. of the sulfur–potassium hydroxide reagent. Shake for 15 seconds and carefully note the time of mixing. Treat the standard and a blank, consisting of 1.0 ml. of pure kerosine, simultaneously in the same manner. Prepare samples in duplicate.

Immediately place the stoppered test tubes in a water bath maintained at 30° ± 0.5° C. Allow exactly 73 minutes at this temperature (counted from the time of mixing with the reagents) for color development. Take the tubes out of the bath after this time, add a small amount of Celite filter aid, and filter through paper (Whatman No. 1, or equivalent) into Hellige-Diller colorimeter tubes. The filtration should be complete within 2 minutes. Shake the colorimeter tubes quickly before placing them in the colorimeter, to assure uniform temperature within the liquid and to avoid convection currents. Exactly 75 minutes after mixing with the reagents, set the photocolorimeter to 100% transmittance with the blank and read the per cent transmittance of standard and test solutions in the usual manner, using a 560 mμ filter.

Correction for Extract's Own Color. Place exactly 1.0 ml. of the pyrethrum extract to be tested in another 24 × 150 mm. test tube and add exactly 3 ml. of pure carbon tetrachloride, followed by exactly 3 ml. of pure (without sulfur) anhydrous ethanolic potassium hydroxide. Shake for 15 seconds and place in the water bath at 30° for 73 minutes. Treat the standard and a blank, consisting of 1.0 ml. of pure kerosine, simultaneously in the same manner.

As before, take the tubes out of the water bath after 73 minutes, counting from the time of mixing with the reagents, filter through paper with some Celite, and set the photocolorimeter to 100% transmittance with the blank 2 minutes after the tubes are taken out of the water bath. Read the transmittance of the sample and standard as usual, exactly 75 minutes after mixing with the reagents.

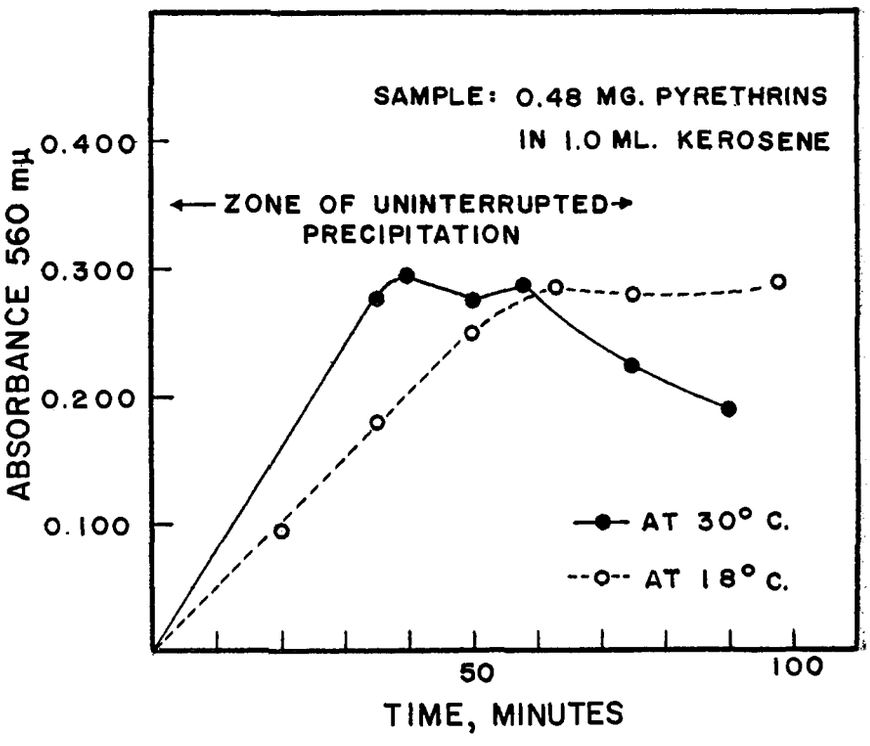
Calculation of Results. Calculate the absorbances by taking the logarithm of the reciprocal of the corresponding transmittances. Correct for the color absorbance of the extract, by subtracting this value from the corresponding absorbances of the analysis solutions of standard and test sample. Use this corrected absorbance to calculate the results by direct proportion between the unknown and the standard.

A typical result on the Hellige-Diller colorimeter, using a 560 mμ filter, would be a reading of 61.5% transmittance for the standard solution containing 0.50 mg. of pyrethrins per ml. This transmittance reading corresponds to an absorbance of 0.211. If the standard solution's own color transmittance reading is 99%, which corresponds to an absorbance of 0.004, the corrected absorbance of the standard is 0.211 − 0.004 = 0.207. Assuming the solution under test gave a transmittance reading of 45.5%, with a color correction reading of 98%, its corrected absorbance is calculated as 0.333, and the final calculation is simply: $x = 0.50(0.333)/0.207 = 0.81$ mg. of pyrethrins per ml. in the solution of the sample.

Discussion of Method

Solubility of Flowers of Sulfur Some lots of flowers of sulfur do not dissolve completely in the carbon tetrachloride at the concentration recommended for preparation of the reagent. If this is the case with the available supply of sulfur, a quantity of soluble sulfur may be prepared by extracting flowers of sulfur with carbon tetrachloride in a Soxhlet apparatus and diluting the extract, after determination of total solids, to the desired concentration of 2.5 grams per liter. The Soxhlet ex-

Figure 1. Effect of time and temperature on color development



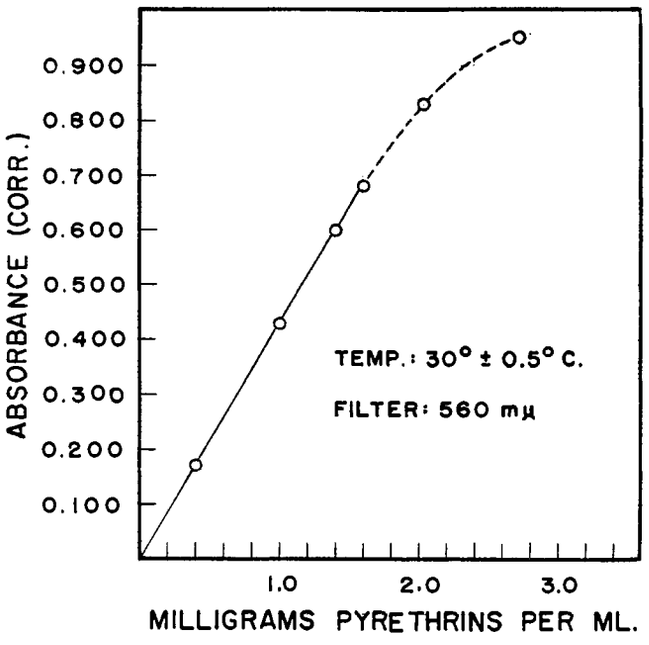


Figure 2. Standard curve for 560 mμ filter

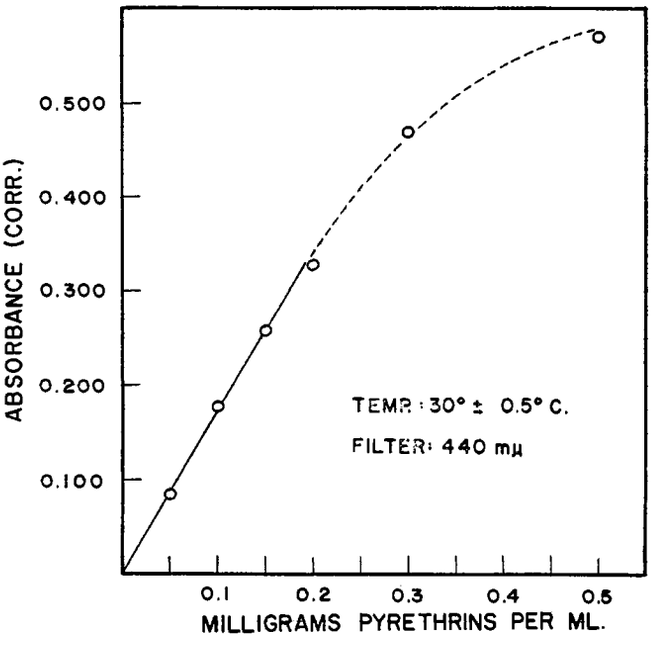


Figure 3. Standard curve for 440 mμ filter

traction can also be replaced by simple shaking of an excess of flowers of sulfur with carbon tetrachloride, decantation of the solution, determination of its total solids content and dilution to the desired strength.

Effect of Time and Temperature on Color Development The color intensity, when developed at 30° C., reaches two maxima, one after 39 minutes and one after 58 minutes (Figure 1, solid line). This is followed by a continuing fall in intensity. These measurements were made within 2 seconds of filtration.

Uninterrupted precipitation during the first 70 minutes of color development makes accurate colorimetric measurements impossible in this region. After the first 70 minutes of color development, the filtered solutions stay clear and free from precipitation for a sufficient time (5 to 8 minutes) to permit accurate colorimetric measurements.

It is important that all the test tubes used in one set of determinations be of the same diameter, to assure a similar rate of warming for all the solutions.

Color development at 18° C. (room temperature in Quito; Figure 1, broken curve) is impractical because of uninterrupted precipitation during the test, making accurate colorimetric measurements impossible.

Effect of Proportion of Pyrethrins in Sample Four samples, containing pyrethrins I and II in different proportions, were analyzed by the colorimetric method. The results (Table I) show that, as would be expected, a sample with predominantly pyrethrins II gives a slightly lower color reading than a sample with predominantly pyrethrins I.

Concentration Range And Precision As shown in Figures 2 and 3, the relationship between concentration and color intensity follows Beer's law in the range up to about 1.4 mg. of pyrethrins per ml. when the 560-mμ filter is used, and up to about 0.2 mg. of pyrethrins per ml. when the 440-mμ filter is used. Above the latter concentrations there is a gradual deviation from the straight-line function. For optimum precision the range from 0.3 to 1.2 mg. per ml. is recommended for the 560-mμ filter, and the range from 0.05 to 0.15 mg. per ml. is recommended for the 440-mμ filter.

Table III. Effect of Synergists and DDT on Colorimetric Determination of Pyrethrins

Sample	Pyrethrins, Mg./Ml. Kerosine	Colorimetric Measurement (Absorbance at 560 Mμ)
1	0.320	0.256
2	0.320 plus 4.6 mg. piperonyl butoxide[a]	0.257
3	0.320 plus 4.6 mg. synergist MGK-264[b]	0.257
4	0.320 plus 4.6 mg. sulfoxide[c]	0.256
A	0.464	0.222
B	0.464 plus 50 mg. DDT[d]	0.223

[a] U. S. Industrial Chemicals Co.
[b] McLaughlin-Gormley-King Co.
[c] S. B. Penick and Co.
[d] Dichlorodiphenyltrichloroethane, Du Pont technical, settling point 89° minimum.

The pyrethrin content of the standards used for these measurements was determined by the ultraviolet spectrophotometric method (*6*).

Specificity of Test And Interfering Substances Acetaldehyde develops the color; benzaldehyde and acetone do not react positively. The color developed by some compounds related to pyrethrins is given in Table II.

Compounds likely to be found with pyrethrins in commercial insecticides, like some synergists and DDT, were tested for possible interference with the quantitative test for pyrethrins. The results (Table III) show that these compounds do not affect the intensity of the color developed by the pyrethrins, at the concentrations normally encountered in commercial products.

Application to Pyrethrum Flower Analysis

Kerosine, recommended as the solvent for the sample, originally was used so that the method would be readily applicable to the usual type of oil-base insecticides. This solvent has also been found satisfactory for the analysis of pyrethrum flowers, as well as insecticidal dusts. (These observations refer to the kerosine produced by the Anglo-Ecuadorean Oilfields, Ltd., La Liberated, Ecuador.) The ground flowers are extracted in a Soxhlet apparatus with petroleum ether, for 6 hours at 40° to 45° C. The petroleum ether is removed in vacuo from an aliquot of the extract, and the solvent-free residue is dissolved in and diluted with kerosine to obtain a solution containing between 0.2 and 1.0 mg. of

pyrethrins per ml. Exactly 1 ml. of this solution is then taken for colorimetric determination.

The colorimetric method can also be applied directly to solvent-free extracts, by dissolving them in 3 ml. of the sulfur–carbon tetrachloride reagent, without the presence of kerosine. Exactly 3 ml. of the sulfur–potassium hydroxide reagent are then added, the time of addition is carefully noted, and the samples are put in the constant temperature water bath, and filtered and read in the same manner as the kerosine extracts. A solvent-free extract of known composition must be used as a standard. The blank in this case consists of 3 ml. of the sulfur–carbon tetrachloride reagent plus 3 ml. of the sulfur–potassium hydroxide reagent.

Acknowledgment

The authors gratefully acknowledge the helpful assistance of R. B. Stoddard, Herman Wachs, and of Howard A. Jones, U. S. Industrial Chemicals Co. Special thanks are also expressed to W. L. Johnson, U. S. Industrial Chemicals Co., for several suggestions for improvement of the present method, as well as for some additional data.

The pyrethrum flowers were obtained through the courtesy of Kaj and Poul Arends, Ecuadorean-American Pyrethrum Co.

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Received for review December 9, 1953. Accepted May 11, 1954. Presented at the Symposium on Analytical Methods, Insecticide Analysis Committee, Chemical Specialties Manufacturers Association, December 6, 1953.

CITRUS WASTE UTILIZATION

Microbiological Production of Riboflavin and Citric Acid from Citrus Molasses

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Fermentation has frequently been employed as a method for utilizing agricultural wastes and by-products, which in the past have mainly been fermented to products of relatively low value, like yeast and solvent alcohols. To investigate the possibility of converting residues to more valuable materials, the fermentation of citrus molasses to citric acid and riboflavin was studied. Although unsatisfactory, even when greatly refined, for citric acid production, citrus molasses served very well as a substrate for riboflavin production by *E. ashbyii* (NRRL 1363). It was necessary to clarify the molasses and supplement with small amounts of a commercial yeast derivative to obtain maximum yields (over 0.7 gram per liter) in 7 to 9 days. Although the method is not expected to be competitive with existing primary fermentations for riboflavin production, application to the enrichment of citrus molasses for feeding seems practicable.

CITRUS FRUIT PROCESSING, for either canning or concentrate production, yields two major by-products—pulp and press juice. Waste peel, rag, and seeds, with any residual juice, are shredded, limed, and pressed to give a highly fermentable "press water" and a solid "citrus pulp." The amounts and compositions of these fractions vary greatly with the type of fruit, locality, and treatment used, but in general from half to two thirds of the fruit processed appears as primary waste products. In a series of comprehensive reviews on citrus wastes, Von Loesecke (*28, 29*) has presented a very complete summary of the origins and disposition of these wastes, including methods for their conversion to useful derivatives.

The disposition of these materials has become a real problem with the rapid expansion of citrus processing operations in Florida, Texas, and California. Early methods of disposal were often wasteful and ineffective and, with the rapid increase in waste volumes, a potential public health hazard was recognized. Present practice is to dry the solid pulp portion and evaporate the unstable press water to a "citrus molasses" sirup containing about 70% solids. Both molasses and pulp are used now mainly as ingredients of animal feed. A description, with flowsheet, of the citrus pulp and molasses process used by Sunkist Growers, Inc., was recently presented (*6*). The molasses produced was somewhat higher in protein (4 to 6%) but otherwise similar to the composition ranges for this product usually reported. Characteristic analyses for Florida citrus molasses have been presented (*7*), but the composition may vary over rather wide limits (*6, 13, 28, 29*).

Citrus molasses (or the original press water), with its high sugar content, is a potential raw material for fermentation, and a variety of microbiological processes for utilizing this material have been proposed (*28*). However, experimental

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