methyl mercuric chloride per ml. of water. The spectrophotometric curve for the methyl mercury dithizonate has a minimum at 480 mμ, which is 10 mμ shorter than the mercury dithizonate.

The commercial formulation in common use, Panogen, is 2.2% methyl mercuric dicyandiamide and contains a red dye. This dye interferes by masking the color reaction at the pH used in the ethyl and phenyl determination. At a pH above 5.5, the red dye becomes colorless and therefore does not interfere in the procedure described.

One thousand micrograms of copper, cobalt, cadmium, iron, lead, nickel, silver, zinc, bismuth, and mercury (II) do not interfere in the determination. However, when large amounts of these ions are present, one drop of the chloroform phase should be held back in transferring to the second separatory funnel, so that none of the interphase will slip through. The accuracy of the procedure is within 3% or 2 γ, whichever is the larger (Table III).

Table III. Accuracy of Procedure

$CH_3HgC_2N_4H_3$, γ/Ml.	
Calcd.	Found
28	28
44	42
55	54
88	88
110	110
133	133
176	177
220	225
264	263
308	312
440	439

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FUNGICIDE DETERMINATION

Quantitative Determination of Biphenyl in Citrus Fruits And Fruit Products by Means of Chromatostrips

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Biphenyl-treated collars, liners, and pads are currently being used by the citrus industry to prevent molding during shipping and storage of citrus fruits. The possibility that the biphenyl vapors will be absorbed by the fruit has created a need for an analytical method for determining the amounts of biphenyl in the fruit and in processed citrus products. A procedure has been developed to separate the biphenyl from interfering citrus oils by chromatographing on chromatostrips. Two methods of quantitatively measuring the amount of biphenyl on the strips are presented. A visual method makes use of the point of minimum detection of the biphenyl spot under ultraviolet light. In the second method, the biphenyl spot is removed from the strip and eluted with alcohol. The concentration of biphenyl is then measured in an ultraviolet spectrophotometer at 248 mμ. Added biphenyl has been determined in citrus juices in as low a concentration as 0.1 p.p.m. and in citrus peel as high as 600 p.p.m. These methods are well suited to determine the amounts of biphenyl absorbed by citrus fruits during storage or shipment in a biphenyl atmosphere, and amounts occurring in processed citrus products.

THE USE OF BIPHENYL-TREATED COLLARS, liners, and pads for the prevention of molding during shipping and storage of citrus fruits in fiberboard cartons has created a need for an analytical method for the determination of this compound in juice, peel, whole fruit, and processed citrus products.

Tomkins and Isherwood's (*9*) colorimetric method does not have the accuracy required, because of the partial destruction of biphenyl by the sulfuric acid treatment. Steyn and Rosselet (*8*) used an ultraviolet absorption method by applying a correction factor for the absorption caused by the citrus oils. The correction factor was obtained from biphenyl-free fruit, but the accuracy of this method is open to question, as the ultraviolet absroption of orange oil varies with individual fruits (*2*). Knodel and Elvin (*5*) have developed an infrared method for the determination of biphenyl in treated fiberboard cartons where citrus oil is not a factor.

A rapid method is needed capable of detecting biphenyl in as small an amount as 1 p.p.m. in juice, peel, or whole fruit of all varieties of citrus fruits. To achieve this purpose, a chromatographic method was developed for separating the biphenyl from interfering citrus oils by using the chromatostrips of Kirchner, Miller, and Keller (*4*).

These are glass strips coated with an ultraviolet fluorescing adsorbent which is held in place by a suitable binder. The coated strips are used as a microchromatographic column in a manner analogous to paper chromatography, and after development of the chromatogram and removal of excess solvent the strips are examined under ultraviolet light to locate the biphenyl spot.

Two methods of measuring the amount of biphenyl in the spot are presented.

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One is a visual method in which different concentrations are applied to find the one giving the minimum detectable amount of biphenyl. It is rapid and useful for field work wherever an ultraviolet spectrophotometer is not available. In the other method, the chromatographed material is eluted and measured in an ultraviolet spectrophotometer at 248 mμ.

Equipment and Materials

Clevenger distillation apparatus (*10*) modified by replacing the regular 2-ml. receiving buret with a 10-ml. buret constructed from a 10-ml. pipet graduated in 0.1 ml.

Boiling flask, 3-liter, with standard-taper neck.

Electric heating mantle.

Pipets, 0.01-ml.

Volumetric flasks, 1-, 2-, and 3-ml.

Syringe microburet (optional for the visual method).

Ultraviolet lamp. Lamps with main output at 365 mμ are unsatisfactory; a short-wave-length lamp (253.7 mμ) is essential.

Ultraviolet spectrophotometer.

n-Heptane or iso-octane, free from aromatic and other ultraviolet-absorbing impurities.

Low-boiling (30° to 60° C.) petroleum ether, free of ultraviolet-absorbing impurities.

Reagent-grade silicic acid.

Zinc-cadmium sulfide.

Zinc silicate.

Starch (Clinco 15 modified starch), or 2 to 1 mixture of ordinary cornstarch and Superior AA tapioca flour.

A mechanical blender.

Water-soluble grease (*7*).

Test tubes, 18 × 150 mm.

Mechanical draft oven.

Glass strips, 13 × 136 mm.

Citrus juice reamer, hand or mechanical.

Glass-stoppered flasks or bottles for oil samples, 10-ml.

Preparation of Materials

n-Heptane or iso-octane (pure grade) is further purified by fractional distillation, followed by chromatographic purification by the method of Graff and associates (*7*).

Petroleum ether (reagent grade) is purified in the same manner as the heptane or iso-octane.

Chromatostrips are prepared by the method originally published (*4*), except that either Clinco 15 modified starch or a mixture of cornstarch and tapioca flour is substituted for the Amioca starch.

Preparation of Sample

The sampling procedure varies. For juice, a 1500-ml. sample is taken. If it is desired to analyze a single fruit, it is ground with 200 ml. of water in a blender to a slurry which is then diluted to 1500 ml. To analyze peel, 100 grams of peel are ground to a slurry with 100 ml. of water and then diluted to 1500 ml.

The 1500-ml. sample is placed in the 3-liter boiling flask fitted to the modified Clevenger buret. (The regular 2-ml. Clevenger buret cannot be used, because with the amount of solvent used the condensed water tends to collect in drops and push the solvent over into the distilling flasks.) One milliliter of *n*-heptane or iso-octane is placed in the 10-ml. receiving buret, and the contents of the flask are gently boiled for 3 hours without foaming or bumping. After distillation, the condenser and upper part of the still head are rinsed with 1 ml. of the solvent, and the total volume of heptane or iso-octane solution, containing the citrus oil and the biphenyl, is read and drained into a flask.

Visual Method

Portions of the heptane-oil-biphenyl solution are diluted with *n*-heptane to make dilutions of 1 to 10 and 1 to 100. With a 0.01-ml. pipet, portions of the undiluted and diluted solutions are spotted at the origin on a series of chromatostrips. A syringe microburet (*6*) is convenient for spotting the strips and decreases the number of dilutions needed.

The strips are placed in test tubes containing 1 ml. of purified petroleum ether (boiling point 30° to 60° C.) and stoppered. When the solvent reaches (by capillarity) a line of 5 cm. above the origin (approximately 7 minutes), the strips are removed and the excess solvent is evaporated in the air (traces of solvent tend to obscure the ultraviolet-absorbing spot). The strips are then examined under the ultraviolet lamp. If biphenyl is present in high enough concentration, it will appear as a dark spot on the fluorescent background. A series of strips containing 0.01-ml. or multiples of 0.01-ml. portions of the several dilutions should be prepared in this manner. For example, from the original solution 0.005-, 0.01-, 0.02- and 0.05-ml. portions are spotted on individual strips; from the 1 to 10 dilution 0.005-, 0.01-, and 0.02-ml. portions are applied; and from the 1 to 100 dilution similar amounts are spotted. When amounts greater than 0.01 ml. are used, separate 0.01-ml. portions are applied on the strip and the solvent is removed by an air stream before the next 0.01 ml. is added.

After development, the strips are examined under ultraviolet light and the strip is selected which just shows a faint but definite dark biphenyl spot. For example, if 0.005, 0.01, 0.02, and 0.03 ml. of a given solution are spotted on strips and, after development, biphenyl spots are visible on all strips except the 0.005 one, then additional strips are run with amounts between 0.005 and 0.01 ml. to find the strip with the minimum detectable amount. In this laboratory this was found to be 0.55 γ of biphenyl. (As this may vary with individuals, each analyst should determine this value for himself.)

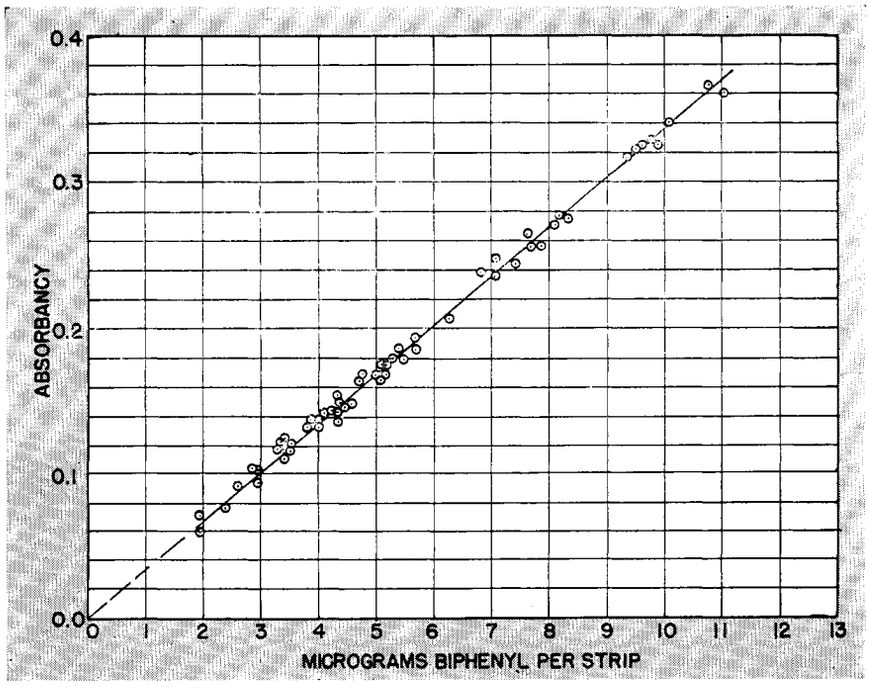
Calculation. Milligrams of biphenyl $= 5.5 \times 10^{-4} \frac{V}{MC}$

where

V = ml. of heptane or iso-octane solution in receiving buret
M = ml. of sample spotted on strip
C = dilution factor, 0.1, 0.01, etc.

Figure 1. Spectrophotometric standard curve for determination of biphenyl

λ = 248 mμ. 10 mm. cell



Spectrophotometric Method

In order to increase the accuracy of the method, a spectrophotometric procedure was adopted for measuring the amount of biphenyl after it is separated by chromatography on chromatostrips. In this case, prior to the application of the biphenyl solution to the strips, the latter are washed by placing in stoppered test tubes containing 1 ml. of 95% ethyl alcohol and allowing the solvent to travel up the strip. They are then dried at 85° C. for 4 minutes in a mechanical convection oven, and after cooling in a desiccator they are ready for use. This is done just prior to the analysis, in order to decrease the blank by removing impurities adsorbed from the air.

For the spectrophotometric method the biphenyl solution is applied to the chromatostrip by means of a syringe microburet (*6*), capable of delivering 0.1 microliter of solution. Both the ground-glass joint of the delivery tip and the plunger of the syringe are lubricated with a water-soluble grease (*7*) in order to avoid evaporation losses.

The strips are developed in petroleum ether (30° to 60° C.) which has been freed of ultraviolet-absorbing materials. In this case development is allowed to proceed until the solvent has traveled 10 cm., in order to get a greater separation of the biphenyl from the other ultraviolet-absorbing materials in the citrus oil. The strips are then carefully dried in an air stream until the solvent odor disappears, and under ultraviolet light the limits of the biphenyl spot are marked. These spots are then transferred to a sintered-glass funnel by scraping off a standard area 22 mm. long the full width of the strip, with the biphenyl spot located at the center of the area. To avoid transfer losses, this is best accomplished by use of a special cone-shaped funnel, 40 mm. in diameter at its widest part with a 10-mm. sintered-glass disk at the bottom. The biphenyl is eluted with 95% alcohol, the eluate being caught directly in a 3-ml. volumetric flask, which is a convenient volume for filling the 10-mm. quartz cells. After making up to volume, the ultraviolet absorption of the eluate is measured in the spectrophotometer at 248 mμ, using 95% alcohol as the blank. The reading is then corrected using a blank obtained by running several strips through the procedure without biphenyl prior to each day's run. The strips are run in triplicate for each analysis and the average density value is used. From the corrected density value the amount of biphenyl on the strip is determined from a standard curve (Figure 1).

Results and Discussion

These methods are based on the fact that the oxygenated constituents in citrus oil are tightly adsorbed at the origin when petroleum ether is used as the solvent (*3*). Biphenyl, a hydrocarbon, moves and has an R_f value of 0.45 (on the alcohol-washed strips), while the citrus oil hydrocarbons are less tightly adsorbed and move close to the top of the strip (R_f 0.95). In this chromatographic separation, therefore, the biphenyl is well separated from the other constituents of the sample and may be observed and eluted without interference.

For the visual method the minimum limit of detection on a strip has been found to be 0.55 γ of biphenyl, but the concentrating effect of steam distillation in the Clevenger apparatus permits 0.05 p.p.m. to be detected in citrus juice when a 1500-ml. sample is used, or 1.5 p.p.m. with a 50-ml. sample of juice from a single fruit. With 19 samples ranging from 0.5 to 575 p.p.m., the average error was found to be 11.3%. The tendency toward larger errors is increased at higher concentrations, because of the large dilution factors, which tend to amplify the errors in reading the minimum visible concentration. Table I shows the recovery of added biphenyl by the visual method.

For the recovery experiments in the juice and whole fruit, aliquots of a biphenyl-heptane solution were added to the sample, and for the peel samples weighed amounts of biphenyl were added directly to the ground peel.

Figure 1 shows the spectrophotometric standard curve, which was prepared by adding known amounts of biphenyl to various citrus samples. This curve includes determinations on lemons, limes, grapefruit, Valencia oranges, and navel oranges as peel, juice, and whole fruit samples to which varying amounts of biphenyl were added. It covers amounts as low as 0.1 p.p.m. in the juice up to 600 p.p.m. in peel samples. The average error is ±2.8% with a maximum error of 9.3% in 57 analyses. Of these 57 analyses only 7 were over 5% in error.

Table I. Recovery of Added Biphenyl from Citrus Products Determined by Visual Analysis

Product	Biphenyl Added, p.p.m.	Biphenyl Added, mg.	Biphenyl Found, mg.	Biphenyl Difference, mg.	Biphenyl Error, %
Valencia orange juice	0.5	0.75	0.68	0.07	9.3
	0.5	0.75	0.74	0.01	1.3
	1.0	1.50	1.60	0.10	6.7
	1.25	1.68	1.89	0.21	12.5
	10.0	15.0	15.6	0.60	4.0
Lemon juice	0.1	0.15	0.17	0.02	13.3
	0.5	0.75	0.64	0.11	14.7
	0.5	0.75	0.85	0.10	13.3
	1.0	1.50	1.60	0.10	6.7
	1.0	1.50	1.76	0.26	17.3
	1.1	1.65	1.57	0.08	4.8
	1.1	1.65	1.87	0.22	13.3
	2.0	3.00	3.18	0.18	6.0
	4.0	6.00	7.00	1.00	16.7
	5.0	7.50	6.48	1.02	13.6
	5.0	7.50	8.50	1.00	13.3
Grapefruit juice	1.08	1.62	1.38	0.24	14.8
Orange peel	567	56.7	71.5	14.8	26.1
Lemon peel	575	57.5	53.4	4.1	7.1

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