Determination of Piperonyl Butoxide by Gas Chromatography

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A gas chromatographic method has been developed for the determination of piperonyl butoxide in technical piperonyl butoxide. The sample is dissolved in a solvent containing a definite concentration of dioctyl phthalate as an internal standard, and the piperonyl butoxide is determined by relating the area of the piperonyl butoxide peak to that of dioctyl phthalate. The method is precise and results are slightly lower than those of the AOAC colorimetric method. The procedure is also applicable to common blends of pyrethrins and piperonyl butoxide.

Gas chromatographic studies of insecticidal concentrates containing piperonyl butoxide (the butyl carbitol ether of 3,4-methylenedioxy-6-propylbenzyl alcohol) indicated the feasibility of a gas chromatographic method for the determination of this widely used synergist for pyrethrins and related insecticides.

The AOAC method (1965) is the most common procedure used for determining piperonyl butoxide in the technical grade material, insecticidal concentrates, or finished formulations. Other methods applicable to pyrethrum synergists which contain the methylenedioxyphenyl group have been reported by Beroza (1956) and Blum (1955). The AOAC method was developed by Jones *et al.* (1952) and is based on the blue color produced by heating piperonyl butoxide with tannic acid in the presence of phosphoric and acetic acids. Interferences which have been reported are pyrethrins, allethrin, alkylated naphthalenes, cyclohexanone, and high-boiling hydrocarbons (Allen *et al.*, 1962; Jones *et al.*, 1952). Jones *et al.* (1952), Beroza (1956), Blum (1955), and Velenovsky (1964) report that the method is specific for piperonyl butoxide in technical material. Allen *et al.* (1962), however, using a similar reagent except with a higher concentration of tannic acid, found essentially the same intensity of color for technical piperonyl butoxide as for pure material. Blazejewicz (1966) found 96% piperonyl butoxide in technical material by a modified Jones procedure (World Health Organization, 1956) and only 84.2% after purification by thin-layer chromatography, indicating that impurities, which are determined as piperonyl butoxide, comprise about 12% of the technical material.

The gas chromatography of 3,4-methylenedioxyphenyl derivatives including piperonyl butoxide has been reported by Zielinski and Fishbein (1966), and a modified electron-capture cell has been used for piperonyl butoxide detection by Bruce (1965).

The present study reports a gas chromatographic method for the quantitative determination of piperonyl butoxide in technical material. A comparison is made of the results by this method with those obtained by the AOAC method on technical piperonyl butoxide and on distillation fractions. Results indicate that the method may be applicable to the determination of piperonyl butoxide in common blends with pyrethrins.

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EXPERIMENTAL

Apparatus. The gas chromatograph used was a Barber-Colman, Series 5000, equipped with flame ionization detector and a 2-foot × 8-mm. O.D. glass column packed with 10% Apiezon L on Anakrom ABS, 110- to 120-mesh. The Barber-Colman Chromocorder was equipped with a disk integrator.

Reagents. Pure piperonyl butoxide was prepared by distillation and chromatographic treatment of technical piperonyl butoxide. Technical piperonyl butoxide was distilled using a 12-inch Vigreux column at 0.1-mm. pressure. That fraction distilling between 170° and 172° C. was further purified by liquid chromatography. Ten grams of the distillation fraction was chromatographed on a $1^3/_4 \times 30$ inch column containing 430 grams of silica gel, 60- to 200-mesh, Grace Chemical Co., Grade 950, Code 950-08-08-226. The piperonyl butoxide was eluted with 6% ethyl acetate in heptane, and the eluate was collected in 125-ml. fractions. The piperonyl butoxide was found by thin-layer chromatography in fractions 32 through 71. The pure piperonyl butoxide (9.5 grams) was recovered from these fractions by evaporation of the mixed solvent. The recovered material was colorless and produced only one spot on a thin-layer chromatography plate. Temperature-programmed gas chromatography indicated a purity of 99.7%.

The internal standard solution was prepared by dissolving 30.0 grams of technical grade dioctyl phthalate in *n*-heptane, diluting to 1 liter with heptane, and thoroughly mixing. The dioctyl phthalate did not produce a gas chromatographic peak in the area of the piperonyl butoxide peak even at high sensitivity. It may be necessary to ensure the absence of interfering impurities and restandardize the method (redetermine the factor) if a new lot or source of dioctyl phthalate is used.

The reagents for the AOAC procedure were exactly as described in the official method (Association of Official Agricultural Chemists, 1965).

Procedure. GAS CHROMATOGRAPHIC METHOD. An approximately 0.7-gram sample of pure piperonyl butoxide was weighed into a 25-ml. volumetric flask and diluted to 25 ml. with the internal standard solution, and the mixture shaken thoroughly. (This solution is stable for at least 6 months.) A 0.3-μl. sample of the mixture was injected on the column maintained at 250° C. The injection port and detector were at 280° C. The helium flow rate was 60 ml. per minute. An attenuation of 300 produced about

50% scale deflection. The relative area of the two peaks was determined, and a factor (grams of piperonyl butoxide per area of piperonyl butoxide peak per area of dioctyl phthalate peak) was calculated. The factor should be determined in duplicate and redetermined periodically, especially when a new internal standard solution is prepared. The factor does not change appreciably with slight variations in column or instrumental conditions.

A 0.7-gram sample of technical piperonyl butoxide was weighed into a 25-ml. volumetric flask, diluted with the same internal standard solution, and chromatographed under the same conditions as those of the pure piperonyl butoxide. The ratio of the peak areas was determined in the same manner, and the per cent piperonyl butoxide calculated as follows:

$$\% \text{ piperonyl butoxide} = \frac{\text{factor} \times \text{area ratio} \times 100}{\text{sample wt., grams}}$$

Area ratio =
area of piperonyl butoxide peak/area of DOP peak

COLORIMETRIC METHOD. The AOAC procedure (1965) was followed.

RESULTS AND DISCUSSION

Technical and purified samples of piperonyl butoxide were chromatographed with temperature programming to learn more about the number and volatility of components present and to prove that the method is specific for piperonyl butoxide in technical material. Figure 1 is a chromatogram of a sample of technical material programmed from 140° to 270° C. at a program rate of 6° per minute. The instrument was operated isothermally at 270° C. after the program was complete until all of the components had been eluted. The attenuation was changed during the run to enhance minor peaks and keep major peaks on scale. At least 17 components are eluted before, and three after, piperonyl butoxide. The authors assume that no impurity is eluted simultaneously with piperonyl butoxide. The pure piperonyl butoxide used to standardize the method exhibited only seven very minor peaks at high sensitivity when chromatographed with temperature programming.

Figure 2 shows a typical chromatogram obtained in the gas chromatographic determination of piperonyl butoxide. The piperonyl butoxide is eluted in about 15 minutes and dioctyl phthalate in 20 minutes. Low-boiling impurities are eluted simultaneously with the solvent (heptane). Other impurities do not cause a visible deviation from the baseline when recorded at the same sensitivity as that of the piperonyl butoxide.

The standard deviation of the gas chromatographic method was calculated as 2.0% (relative) from results on 12 replicate determinations on pure piperonyl butoxide. This compares with a relative standard deviation of 3.1% for the AOAC (colorimetric) method as calculated from 12 determinations on a sample of technical piperonyl butoxide. Velenovsky (1960) reported a reproducibility of ±3% by a single operator for the AOAC method.

The effect of sample size on the gas chromatographic method was determined by plotting the sample weight *vs.* the area ratio (area of piperonyl butoxide peak per area of

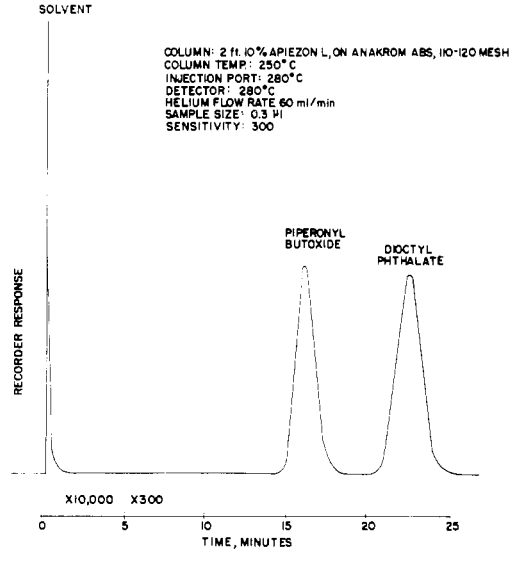

Figure 2. Typical chromatogram of the gas chromatographic determination of piperonyl butoxide

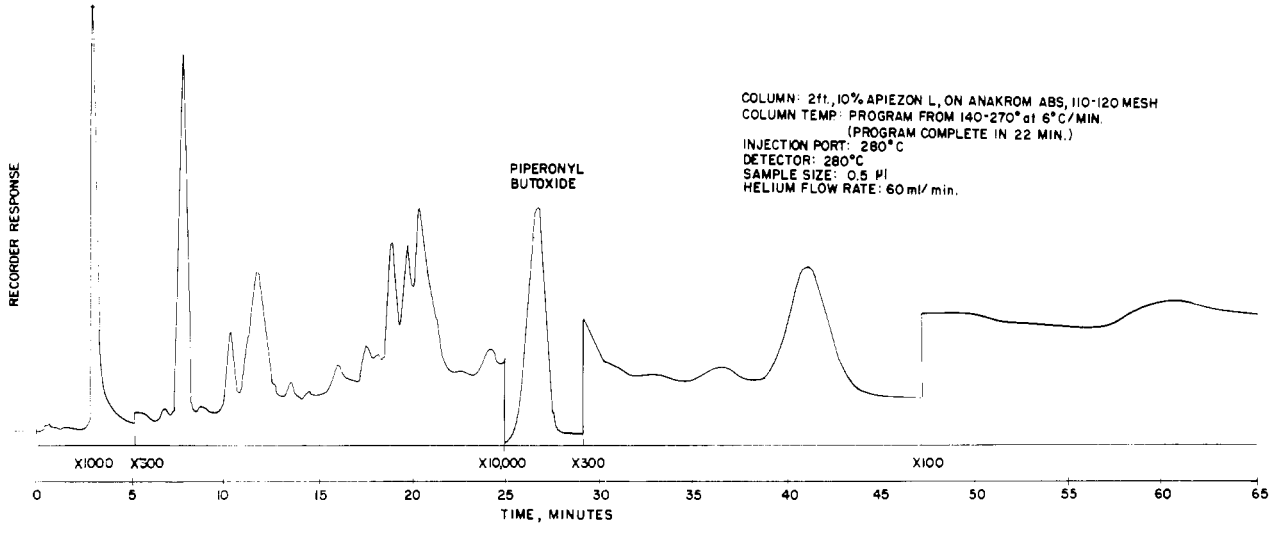

Figure 1. Temperature-programmed chromatogram of technical piperonyl butoxide

dioctyl phthalate peak). A straight-line plot was obtained with sample sizes between 0.3 and 1.0 gram.

The gas chromatographic method has been used in the authors' laboratory for approximately a year. Several samples of technical piperonyl butoxide from two commercial sources have been analyzed by the gas chromatographic and colorimetric methods (Table I).

The values by the colorimetric method average almost 2% higher than those by the gas chromatographic method. To determine possible interference of a related material in the colorimetric method, distillation fractions were analyzed by both methods. Any interfering component should be concentrated in one distillation fraction, and the results by the colorimetric method (relative to the gas chromatographic method) should be higher in that fraction. Furthermore, the agreement between methods should be within experimental error in the pure fractions. Results by both methods are shown in Table II for fractions recovered by distillation of technical piperonyl butoxide at 14-mm. pressure using a 12-inch Vigreux column.

The 200-gram sample before distillation represented 159.6 grams of piperonyl butoxide as determined by the gas chromatographic method or 165.2 grams by the colorimetric method. Assuming no piperonyl butoxide in the residue, 152.3 grams of piperonyl butoxide, or 95.4%, are recovered in the 10 fractions as determined by gas chromatography and 159.1 grams, or 96.3%, by the colorimetric method.

The colorimetric results are consistently higher than those by the gas chromatographic method. The cut in which the presence of an interfering material seems most likely is fraction 10, which distilled adjacently higher than the purest cut. This fraction represents 8% of the original sample and yielded a piperonyl butoxide content 15% higher colorimetrically than chromatographically. This would represent a possible interference of only 1.2% in the total sample. Although the colorimetric results are always higher, the methods are not in serious disagreement, especially in the purer fractions. The variation between methods, however, is consistent and significant. Since both methods are standardized against the same pure material, technical piperonyl butoxide probably contains a small amount of impurity, not easily separated by distillation, which produces a color with the AOAC reagent. Isolation of the suspected impurity and proof of the interference will be the subject of further investigation.

Two other purified samples were analyzed by both methods. Assays of 95.4% by gas chromatography and 98.0% colorimetrically were obtained on a sample purified by single distillation. Corresponding values of 98.6% and 100.6% were obtained on a double-distilled sample.

In order to test the accuracy of the gas chromatographic method over a wide range of piperonyl butoxide content, a series of prepared samples was analyzed. The pure piperonyl butoxide (double-distilled sample) was mixed with butyl carbitol. The data shown in Table III indicate good agreement between the experimental and known values for piperonyl butoxide.

Pyrethrins do not interfere significantly in the gas chromatographic method in a normal piperonyl butoxide to pyrethrins ratio. Piperonyl butoxide is nearly always associated with pyrethrins in insecticidal formulations and often is purchased as a blend with pyrethrins. Pyrethrins interfere with the piperonyl butoxide determination by the AOAC method, and in order to circumvent the interference, the unknown sample is always compared with a standard containing pyrethrins and piperonyl butoxide in the same ratio as that of the sample. A sample containing 36.7% piperonyl butoxide and 11.0% pyrethrins in deodorized kerosine was analyzed by the gas chromatographic method and 37.9% piperonyl butoxide was found. Closer study showed that one of the four components of pyrethrins is eluted simultaneously with piperonyl butoxide and another is eluted with dioctyl phthalate. Since the interferences are compensating at least partially, the effect on the value for piperonyl butoxide is not very significant. This is especially true since the amount of piperonyl butoxide in a common blend is approximately 20 times that of any single component of pyrethrins. Pyrethrins probably will not interfere significantly in most mixtures, but additional work

Table I. Analysis of Technical Piperonyl Butoxide

Lot Number	Piperonyl Butoxide, %: Gas chromatographic	Piperonyl Butoxide, %: Colorimetric
1646-88	81.3	83.0
1269	79.8	82.6
72B	82.4	81.7
975	79.6	81.9
Butacide[a]	87.7	90.8

[a] F.M.C.'s improved deodorized piperonyl butoxide.

Table II. Analysis of Distillation Fractions

Distillation Fraction	Distillation Range, ° C.	% of Total Sample	Piperonyl Butoxide, %: Gas chromatographic method	Piperonyl Butoxide, %: Colorimetric method
Undistilled		100	79.8	82.6
1	Under 115	6.30	<0.02	<0.1
2	115–128	1.35	0.31	0.83
3	128–150	1.15	3.5	3.7
4	150–164	1.65	3.9	5.2
5	164–200	1.60	12.3	13.1
6	200–231	2.00	49.3	54.1
7	231–238	3.85	79.2	84.7
8	238–240	5.95	94.9	99.7
9	240–242	64.0	98.2	101.7
10	242–242	8.35	40.0	45.8
Residue (solid)		3.15	[a]	[a]

[a] Piperonyl butoxide not determined in residue.

Table III. Accuracy of Gas Chromatographic Method

Sample	Piperonyl Butoxide, %: Actual[a]	Piperonyl Butoxide, %: Found
1	74.7	73.5
2	33.6	32.9
3	49.9	49.7
4	66.7	65.5
5	24.7	25.2

[a] Uncorrected for impurities; temperature-programmed gas chromatography indicated that the piperonyl butoxide used to prepare the samples was 99.3% pure.

should be done before applying the method to the quantitative determination of piperonyl butoxide to blends.

No attempt has been made to apply the gas chromatographic method to the determination of piperonyl butoxide in finished formulations. The method could be modified for use in finished formulations if it is ascertained that no interfering materials are present in the formulation.

ACKNOWLEDGMENT

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Correction

IMPORTANCE OF CELLULAR CONSTITUENTS TO COTTONSEED MEAL PROTEIN QUALITY

In this article by W. H. Martinez, L. C. Berardi, V. L. Frampton, H. L. Wilcke, D. E. Greene, and Robert Teichman [J. AGR. FOOD CHEM. **15**, 427 (1967)], the second to the last sentence in the Results and Discussion section on page 432 should read, "It is possible that the presence and absence of the carbohydrate constituents in CM72, CM72-CM, CM72-A are the major reason for the difference between the ϵ-free lysine values of the three unautoclaved meals."

Correction

DETERMINATION OF PECTIC SUBSTANCES IN THE PRESENCE OF DEXTRINS

In this article by M. A. Joslyn and Tung-Shan Chen [J. AGR. FOOD CHEM. **15,** 398 (1967)] in the subcaption to Figure 2, page 401, item 9 should be item 6, and item 7 should be added as follows:

7. Dextrin, 50 mg./liter, no carbazole added