Assay of Barban, 4-Chloro-2-butynyl-*N*-(3-chlorophenyl)carbamate

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A method has been developed to assay 4-chloro-2-butynyl-*N*-(3-chlorophenyl)carbamate, a new postemergence herbicide for the control of wild oats. To effect analysis, barban is separated from its impurities by adsorption elution chromatography using neutral alumina and graded solvents. It is determined in the eluent by its ultraviolet absorbance at 277.5 mμ. Impurities present in the technical material were shown either to elute at different times from barban or to have a discernably different ultraviolet spectrum. Careful control of alumina activity and solvent purity is essential for reliable results. A method of evaluating alumina and of adjusting its activity is described as a part of the procedure. Some problems of solvent purity are discussed. A statistical evaluation of the method shows that the method affords a 1-sigma precision of ±0.45%. Analysis of variance shows that photometry introduces a standard deviation of ±0.14%, while chromatography introduces ±0.43%. The method recovery was shown to be 98.9%.

A METHOD was needed to assay 4-chloro - 2 - butynyl - *N* - (3-chlorophenyl)carbamate (barban), the active ingredient of Carbyne (Spencer Chemical Co. trade-mark). Carbyne, an emulsifiable concentrate containing 1 pound per gallon of barban, is a new selective postemergence herbicide for wild oats (*2, 3*). To show the compounds that had to be dealt with in the analysis, the synthesis route and the two main by-products are shown in Figure 1. 3-Chlorophenylisocyanate is reacted with 2-butyn-1,4-diol to form 4-hydroxy-2-butynyl - *N* - (3 -chlorophenyl)carbamate (hydroxycarbamate), an intermediate, which, in turn, is chlorinated with thionyl chloride to form barban. By-products may be formed by reactions of the original isocyanate with water to form *N,N'*-bis(3-chlorophenyl)urea and with the intermediate hydroxycarbamate to form 2-butynylene bis-*N*-(3-chlorophenyl)carbamate (biscarbamate). These two materials, as well as the intermediate hydroxycarbamate, are the main impurities in the product. Traces of solvent may also be present.

The method used initially to assay recrystallized barban was based on determination of alkyl chlorine by formation of quaternary ammonium salt with monoethanolamine (*4, 5*). This method, however, could not be used for technical material since other components containing alkyl chlorine were present in the commercially produced material. A colorimetric procedure based on the determination of 3-chloroaniline after diazotization and coupling with *N*-(1-naphthyl)ethylenediamine was developed for decline and residue studies in plants (*6*). This method was not suitable for assay since it was not specific for barban. Spectrometric methods were not directly applicable because the ultraviolet and infrared spectra of barban are very similar to the spectra of biscarbamate, the major impurity in the product. The infrared spectra of these materials are shown in Figure 2, and the ultraviolet spectra are shown in Figure 3.

In this work a method was developed to determine barban directly. Barban is separated from its impurities by adsorption elution chromatography and is determined in the eluent by ultraviolet spectrometry.

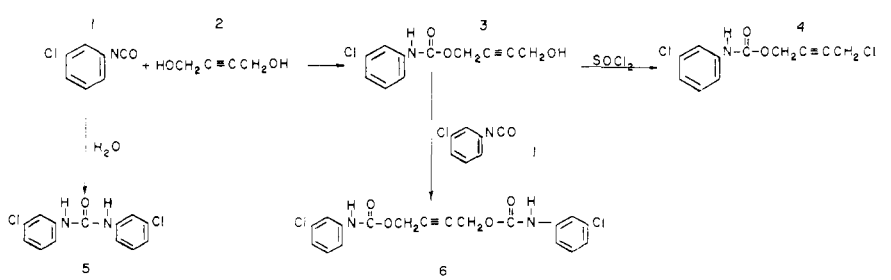

Figure 1. Barban synthesis and by-products

1. 3-Chlorophenylisocyanate. 2. 2-Butyn-1, 4-diol. 3. 4-Hydroxy-2-butynyl-*N*-(3-chlorophenyl) carbamate. 4. 4-Chloro-2-butynyl-*N*-(3-chlorophenyl)carbamate. 5. *N,N'*-bis(3-chlorophenyl)-urea. 6. 2-Butynylene bis(3-chlorophenyl)carbamate

Experimental

Apparatus and Reagents. A Cary Model 14, ultraviolet recording spectrophotometer equipped with log absorbance slide wire was used for all photometric measurements. Silica cells with 1-cm. and 2-cm. path lengths (Pyro Cell, Inc.) were used. The chromatographic columns, 1 cm. in diameter × 50 cm. in length, were made in this laboratory and are shown in Figure 4.

Primary eluting solution was prepared by mixing one volume of ethylene dichloride with two volumes of *n*-hexane. To be sure that this solvent would not be photometrically changed by its passage through the analytical column, the mixture was passed through a 2.5 × 60 cm. column of activated alumina before use.

Barban eluting solution was prepared by mixing one volume of ethylene dichloride with one volume of *n*-hexane. This mixture was also passed through a 2.5 × 60 cm. column of activated alumina.

Ethylene dichloride used to prepare the eluting solutions was distilled and spectrally clear between 260 and 320 mμ when scanned *vs.* water. Hexane was spectro grade.

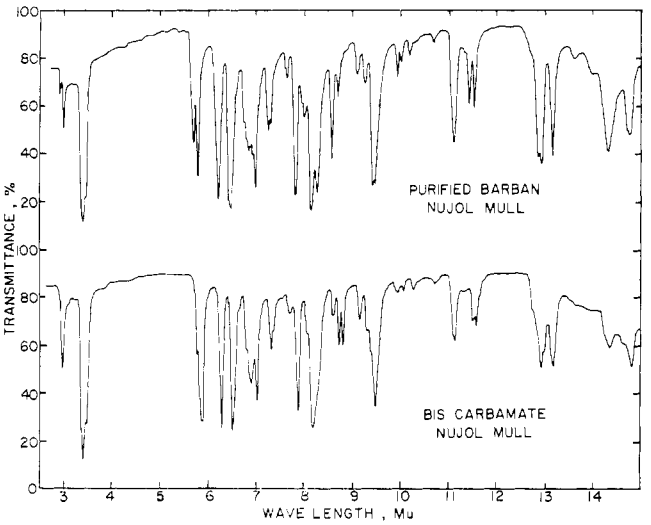

Figure 2. Infrared spectrum of barban and biscarbamate

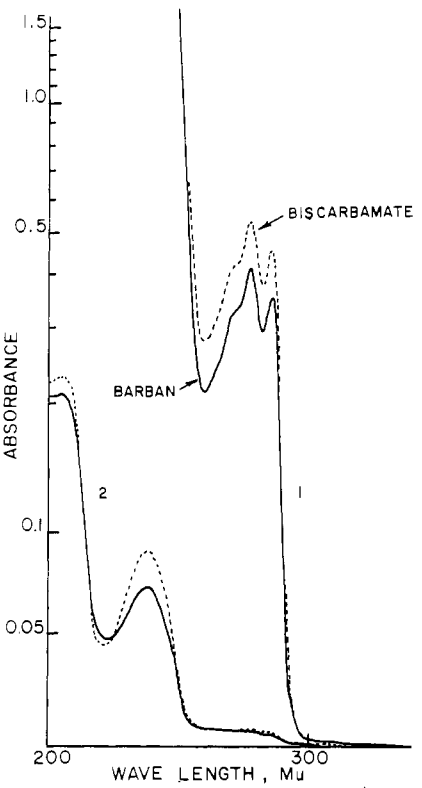

Figure 3. Ultraviolet spectra of barban and biscarbamate

Solvent. Ethyl alcohol
Absorptivity. Barban, 4.11 liters/gram cm.; biscarbamate, 5.32 liters/gram cm.
Concentration. 1. 0.1 gram/liter
2. 0.001 gram/liter

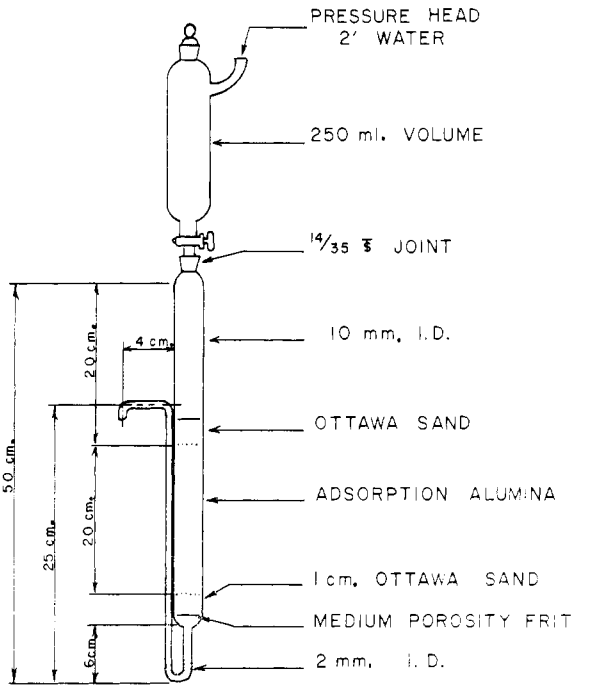

Figure 4. Chromatographic column assembly

Both Fisher 540-A and Woelm alumina were used. The latter (Alupharm Chemical Co., New Orleans, La.) had a reported activity of Brockman Grade I.

Acid-washed Ottawa sand was used in the columns.

Analytical Procedure. Preparation of the Column. The column shown in Figure 4 was filled with *n*-hexane to the level of the drain tip. Washed sand was added to a depth of 1 cm. Dry alumina was poured through a powder funnel into the column and permitted to settle through the hexane. This technique produced columns with desirable operational characteristics which could be prepared with the ease of dry pack columns. When the alumina bridged across the liquid-air interface it was forced down into the liquid with a long slender rod. The column was filled to a depth of 20 cm. and topped with sand. Care was taken to rinse down the sides of the column with *n*-hexane prior to adding sand.

Analysis. Approximately 2 grams of sample were accurately weighed and dissolved in 100 ml. of ethylene dichloride. A 10-ml. aliquot was diluted to 100 ml. with primary eluting solution. A portion of this solution was filtered through rapid paper and a 10-ml. portion of filtrate containing approximately 0.02 gram of barban was loaded on the column.

The column was eluted with 125 ml. of primary eluting solution to remove impurities less strongly retained than barban. The column was then eluted with 220 ml. of barban eluting solution. The eluent was collected in a 250-ml. volumetric flask, diluted to volume with barban eluting solution, the absorbance determined at 277.5 mμ, and the amount of barban present was calculated.

Evaluation of Alumina. Early in the development of this procedure it was found that a specific activity of the adsorbant was needed to allow barban to be eluted separately from its impurities and that the alumina had to be given a preliminary evaluation. This was done by loading the alumina column with 10 ml. of solution containing 0.02 gram of barban. It was then eluted with 125 ml. of primary eluting solution, and five separate 25-ml. portions of eluent were collected. The column was then eluted with 225 ml. of barban eluting solution and nine separate 25-ml. portions were collected. All 14 fractions were scanned from 400 to 200 mμ, and the absorbance read at 277.5 mμ. This represents the maximum absorbance in the benzenoid region for barban. A plot of this *vs.* milliliters eluted resulted in a differential chromatogram as shown in Figure 5.

The alumina activity required for accurate barban analysis by this separation procedure is represented by *A* in Figure 5. As shown, barban should first appear in the eluent after approximately 150 ml. of solution had passed from the column. All of the barban should be removed from the column by the time 325 ml. have passed through the column. If the alumina were too active, the barban would tail badly producing a chromatogram similar to *B*. If it were not sufficiently active, it would elute too soon as shown in *C* and be partly lost in the primary eluent.

Discussion of Development Procedure. A solvent system was needed which would effect solution of a reasonable quantity of technical barban, be spectrally clear, and be sufficiently nonpolar to enable a stepwise increase in polarity to enable separation of impurities. Hexane was chosen as the nonpolar solvent, although it had low solution capacity. For use as the polar solvent ethyl alcohol, ethylene dichloride, and ethyl acetate all exhibited

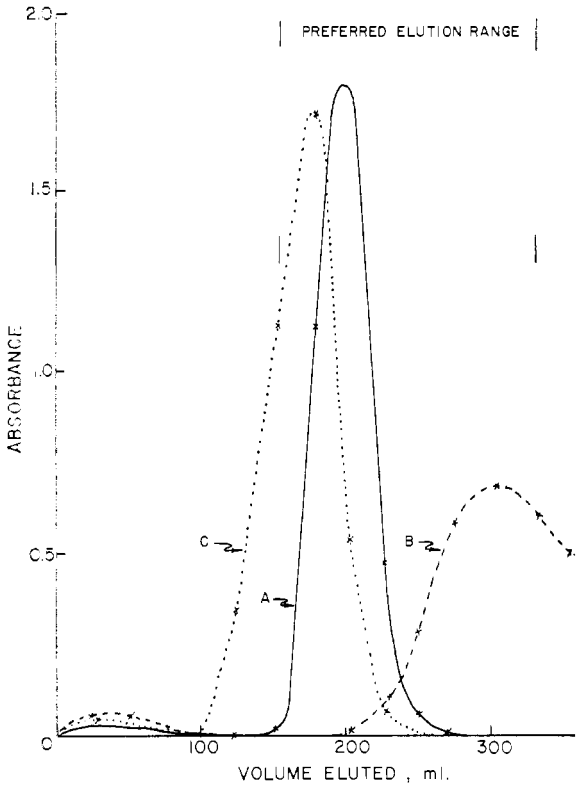

Figure 5. Elution characteristic of Fisher alumina

A. Correct activity with 4.25% water added
B. Too active with 3.50% water added
C. Too inactive with 4.75% water added

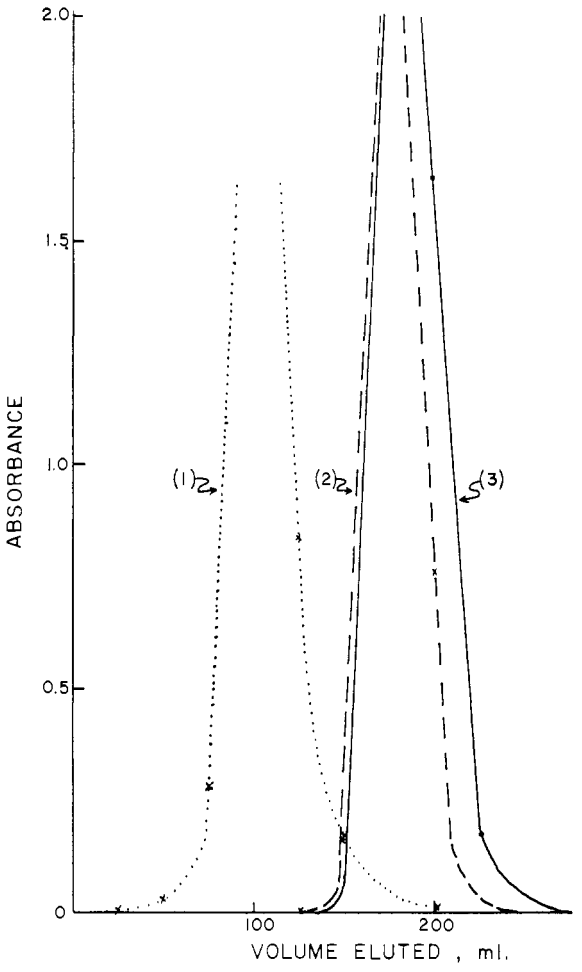

Figure 6. Elution characteristic of Woelm alumina with 2.25% water added

1. Acid alumina
2. Basic alumina
3. Neutral alumina

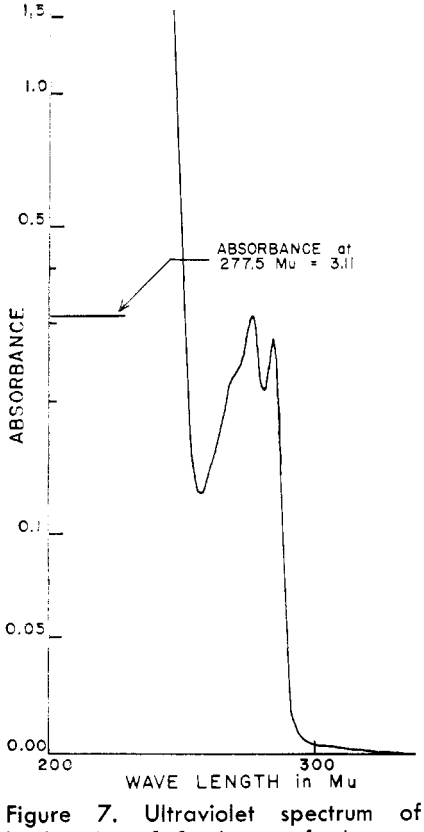

Figure 7. Ultraviolet spectrum of barban in a 1:1 mixture of *n*-hexane and ethylene dichloride

Absorptivity in eluting solution. 3.83 liters/gram cm.

adequate solution capacity. Ethyl alcohol gave the best spectral clarity, but due to its high polarity was not used. Ethylene dichloride was chosen because it provided the best over-all properties. It is spectrally clear above 250 mμ, displays moderate polarity and is an excellent solvent for barban.

A preliminary study was made to determine the proper solvent ratio required to obtain the needed solvent capacity and solution polarity. A 2-gram portion of technical material was taken up in enough ethylene dichloride to effect a nonturbid solution. This was then diluted with *n*-hexane until turbidity was attained. Ethylene dichloride was then added until turbidity disappeared. It was determined that a 100-ml. volume of a mixture of one part of ethylene dichloride to two parts of *n*-hexane could completely dissolve 2 grams of barban and yet not elute it from a 20-cm. column of alumina of proper activity. By successively increasing the amount of ethylene dichloride in the eluting solvent, it was found that a mixture of equal parts of ethylene dichloride and *n*-hexane would effectively elute barban from the alumina. This change in polarity also enabled separation of impurities which are less strongly adsorbed than barban. Consequently, ethylene dichloride and *n*-hexane in the ratio of 1 to 2 and 1 to 1, respectively were chosen as primary eluting solution and barban eluting solution.

To check the eluting solutions a 0.1837-gram portion of recrystallized barban, which had been previously analyzed as 99.4% pure by the alkyl chlorine method, was loaded on a column and eluted as shown in Table I. As may be seen, a 1:2 ratio of ethylene dichloride to hexane was totally ineffective in removing barban, while virtually the total sample was removed by the 2:2 ratio.

A similar procedure was employed to determine elution characteristics of the biscarbamate. It was shown that biscarbamate was not eluted with any combination of ethylene dichloride and *n*-hexane, nor even with pure ethylene dichloride. The biscarbamate was eluted with ethyl alcohol.

The initial work was done with Fisher 540-A alumina. Since the alumina initially used had higher activity than was desired for this separation, it was hoped that proper activity could be attained

Table I. Elution Check by Weight

Fraction	Volume Ratio, EDC[a]-n-Hexane	Amount Eluted Ml.	Gram	%
1	1:2	150	None	...
2	2:2	150	0.1753	96.2
3	3:2	150	Trace[b]	Trace
4	4:2	150	None	...

[a] EDC is ethylene dichloride.
[b] Presence of barban indicated by ultraviolet spectrum of eluent.

Table II. Method Reproducibility

$$S = \frac{\overline{R}}{F}$$

where

S = reproducibility
$\overline{R}$ = average range
F = 1.128 for subgroups of 2
= 1.693 for subgroups of 3

S_1 for subgroups of 2

$$S_1 = \frac{0.50}{1.128} = \pm 0.44$$

S_2 for subgroups of 3

$$S_2 = \frac{0.78}{1.693} = \pm 0.46$$

S_{Method} at 1-sigma level

$$S_{\text{Method}} = \left[\frac{(0.44)^2 + (0.46)^2}{2}\right]^{\frac{1}{2}} = \pm 0.45\%$$

Reference (*1*)

by controlled deactivation through the addition of water. The chromatograms in Figure 5 show results of deactivation by addition of successive increments of water to Fisher alumina. It was soon discovered that this procedure was not practical, since the activity of Fisher alumina varied widely from jar to jar. In some cases, it was found that freshly opened alumina was already too inactive to be used. Because of the variability of the alumina activity from jar to jar, a procedure was employed completely to deactivate and reactivate the alumina, which produced material of somewhat controlled activity. The procedure was cumbersome and the material so produced did not exhibit elution characteristics as desirable as Woelm alumina; hence the procedure is not discussed here.

Woelm acid, neutral and basic alumina were all evaluated with the results shown in Figure 6. Acid alumina retained barban excessively and controlled deactivation with water appeared to be difficult. Basic alumina was found to be similar to neutral alumina in elution. Because of the slight possibility that the basic form might react with some of the organic compounds present, neutral alumina was chosen as the preferred material. The activity of the neutral alumina was consistent from container to container and in every case could be adjusted to the required activity by the addition of 2.25% water.

Table III. Data from Replicate Chromatographings of Barban

Technical Barban 1	2	Range	Recrystallized Barban 1	2	3	Max. range
85.75	85.10	0.65	98.43	98.10	98.10	0.33
85.83	85.02	0.81	97.45	97.78	97.13	0.32
85.67	85.02	0.65	99.03	100.00	99.35	0.97
85.67	84.70	0.97	98.71	99.67	99.03	0.96
86.38	86.86	0.48	98.71	99.51	98.71	0.80
85.24	84.75	0.49	98.15	98.33	98.39	0.24
84.92	84.92	0.00	97.91	98.39	97.91	0.98
85.08	84.75	0.23	98.15	98.36	98.23	0.21
86.60	86.80	0.20	99.46	98.76	98.76	0.70
86.55	87.42	0.87	99.32	98.60	98.84	0.72
86.78	87.33	0.55	99.40	98.84	98.60	0.80
85.90	86.00	0.10	98.88	99.27	100.25	1.37
			99.04	99.27	100.25	1.21
			98.72	99.27	100.16	1.44
Av. range		0.50				0.78

Reproducibility and Accuracy. The method affords a reproducibility of ±0.45% barban. The reproducibility of the method was determined by replicate analyses of solutions of barban. Analyses of solutions were employed because the production material was not homogeneous. The reproducibility was calculated from the average range by use of appropriate factors (*1*) for subgroups of 2, and for subgroups of 3 as shown in Table II. Average range values used in the calculation were obtained from duplicate analyses of 12 samples of technical barban and from 14 triplicate analyses of recrystallized material. The data from these analyses are tabulated in Table III.

All photometric measurements were made in triplicate. By the method shown previously, the reproducibility of the photometric measurement was calculated as ±0.14%. Data from the photometric measurements are tabulated in Table IV.

By an analysis of variance it was now possible to calculate the reproducibility of the chromatographic separation as shown in Table V. This shows that by far the largest error is introduced by chromatographic separation.

Obviously, the accuracy of the method depends primarily upon the purity of barban used as the photometric standard and upon recovery from the column. Pure barban was prepared from technical material by repeated recrystallization from hot *n*-hexane. The recrystallized material was chromatographed twice using a scale-up of the analytical equipment. The barban was crystallized from the partially evaporated eluting solution and recrystallized from *n*-hexane. The alkyl chlorine analysis indicated a purity of 99.78%. The ultraviolet absorptivity at 277.5 mμ of an ethylene dichloride solution of this material was 3.83. The ultraviolet

Table IV. Data from Replicate Photometric Readings

1	2	3	Max. Range
88.54	88.70	89.03	0.49
87.90	88.22	88.22	0.32
83.39	83.39	83.72	0.33
86.49	86.49	86.49	0.00
85.83	85.67	85.67	0.16
85.02	85.02	84.70	0.32
81.51	81.51	81.51	0.00
84.90	84.74	84.74	0.16
86.98	86.98	86.66	0.32
96.31	86.31	86.47	0.16
85.24	84.92	85.08	0.32
84.75	84.92	84.75	0.17
101.60	101.28	101.60	0.32
99.03	98.71	98.71	0.32
100.00	99.67	99.51	0.49
99.35	99.03	98.71	0.64
100.76	100.72	100.72	0.04
98.15	97.91	98.15	0.24
98.33	98.34	98.36	0.02
98.39	97.91	98.23	0.48
99.88	99.96	100.04	0.16
99.46	99.32	99.04	0.14
98.76	98.60	98.84	0.24
98.76	98.84	98.60	0.24
99.87	100.16	99.99	0.29
100.16	100.28	100.25	0.12
100.08	99.99	100.16	0.17
100.13	100.51	100.51	0.38
99.90	99.56	99.56	0.34
99.12	98.95	99.12	0.17
99.77	97.74	99.77	0.03
98.09	98.26	98.26	0.17
Av. range			0.24

$$\frac{0.24}{1.693} = \pm 0.14\%$$

Table V. Analysis of Variance

$V_{\text{Total}} = V_{\text{Chromatography}} + V_{\text{Photometry}}$

$V_{\text{Chrom.}} = (0.45)^2 - (0.14)^2$

$= 2026 \times 10^{-4} - 196 \times 10^{-4} =$
1830×10^{-4}

$\therefore S_{\text{Chromatography}} = V^{1/2}{}_{\text{Chrom.}} =$
$\pm 0.43\%$ at a 1-sigma level

Reference (*1*).

Table VI. Recovery from the Column

Reading	Column 1	Column 2	Column 3	Unchromatographed
1	0.6128	0.6138	0.6138	0.6208
2	0.6173	0.6128	0.6143	0.6213
3	0.6178	0.6143	0.6128	0.6218
Av.	0.6159	0.6136	0.6136	0.6213

Grand av. = 0.6144

$$\frac{0.6144 \times 100}{0.6213} = 98.9\% \text{ recovery}$$

Numbers expressed as absorbance.

Table VII. Analysis of a Barban Mixture

Technical material	72.14%
Recrystallized material	98.46%
Mixture	92.76%

Theoretical value

Technical

1.362 grams × 0.7214 = 0.9825 gram

Recrystallized

$$\frac{4.432 \text{ grams} \times 0.9846}{5.794 \text{ grams}} = \frac{4.3664 \text{ grams}}{5.3489 \text{ grams}}$$

$$\frac{5.3489 \times 100}{5.794 \text{ grams}} = 92.32\%$$

spectrum of the material is shown in Figure 7.

To evaluate the recovery from the column, the absorbance of a solution of purified barban was measured before and after chromatography. The loss of absorbance is then a direct indication of the extent of loss of barban on the column. The results of this evaluation are shown in Table VI. Three aliquots of a solution of purified barban were chromatographed in separate columns and three photometric readings taken in each case. A recovery of 98.9% was realized. The barban was recovered from the eluting solvent and rechromatographed. On the second pass through the column 98.0% recovery was attained.

The accuracy of the method was evaluated by analyzing a synthetic mixture. The results are shown in Table VII. The sample was prepared by adding 1.362 grams of technical material, which analyzed 72.17%, to 4.432 grams of recrystallized material which analyzed 98.46%. The material was mixed in solution and the solvent was evaporated. The sample analyzed 92.76% as compared to a theoretical value of 92.32%.

Interferences. Several impurities potentially present in the technical material were investigated as interferences in the method. The biscarbamate and the hydroxycarbamate are too strongly adsorbed to be eluted with barban. Bisurea is eluted after barban and has an ultraviolet spectrum sufficiently different from barban as to be recognizable as an impurity in the eluent. It is unlikely that 3-chlorophenylisocyanate would be present, but if so it would be converted to a 3-chloroaniline type compound on the chromatographic column. Toluene, present as residual solvent, is eluted before barban and has a discernably different ultraviolet spectrum.

Acknowledgment

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INSECTICIDE ASSAY

Chromatographic Separation of Active Components of Natural Pyrethrins and Their Characterizations

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IN THE COURSE of a study on the metabolic fate of different pyrethroids in insects, methods for isolation of pyrethrins I and II (PI and PII) and cinerins I and II (CI and CII) were developed. Established synthetic procedures, such as synthesis of pyrethroids from appropriate cyclopentadienolone and acids or by conversion of purified semicarbazones (*23*), are tedious and characterized by low yields and other uncertainties. A purely physical process of separation of the four components has been attempted by various workers. Enriched "PI" (mixture of PI and CI) and "PII" (mixture of PII and CII) have been obtained by repeated liquid–liquid partitions (*8, 20, 27*). "PI" and "PII" have also been separated by paper (*19, 29*) and column chromatography (*6, 10, 17, 18, 22*). Ward (*25*) and later Brown and coworkers (*2, 3, 4*) have achieved partial separation of four active components by displacement chromatography. The same method has been tried in our laboratory and gave only partial resolution at best. Results of these workers suggested that by use of a combination or modification of some of these procedures, a more direct separation and purification of the four pyrethroids could be accomplished.

Three colorimetric tests were used for identification and estimation of purity of individual pyrethroids. The colorimetric test developed by Williams and Sweeney (*24, 28*) was used to determine esters of pyrethrolone. PI and PII