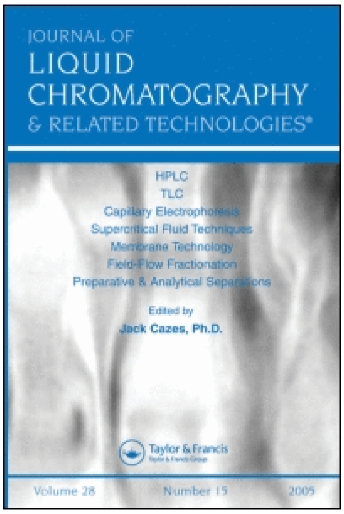


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Chlorflurenol-Methyl and Determination of Octanol-Water Coefficient by Countercurrent Chromatography

N. B. Mandava[a]; Y. Ito[b]; Y. Ma[b]
[a] Mandava Associates, Washington, DC [b] Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, Bethesda, MD

To cite this Article Mandava, N. B. , Ito, Y. and Ma, Y.(1998) 'Separation of Chlorflurenol-Methyl and Determination of Octanol-Water Coefficient by Countercurrent Chromatography', Journal of Liquid Chromatography & Related Technologies, 21: 1, 217 — 229

To link to this Article: DOI: 10.1080/10826079808001951

URL: http://dx.doi.org/10.1080/10826079808001951

J. LIQ. CHROM. & REL. TECHNOL., 21(1&2), 217–229 (1998)

SEPARATION OF CHLORFLURENOL-METHYL AND DETERMINATION OF OCTANOL-WATER COEFFICIENT BY COUNTERCURRENT CHROMATOGRAPHY

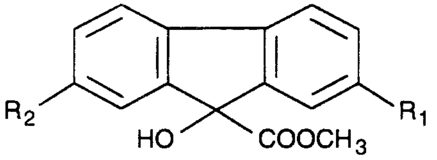
N. Bhushan Mandava,[1] Yoichiro Ito,[2] Ying Ma[2]

[1] Mandava Associates
1625 K Street, N.W.
Washington, DC 20006

[2] Laboratory of Biophysical Chemistry
National Heart, Lung, and Blood Institute
National Institutes of Health
Bethesda, MD 20892

ABSTRACT

Using high-speed countercurrent chromatography (CCC), technical chlorflurenol-methyl was successfully separated into methyl 9-hydroxy-9-fluorenecarboxylate, methyl 2-chloro-9-hydroxy-9-fluorenecarboxylate, methyl 2,7-dichloro-9-hydroxy-9-fluorenecarboxylate. This method can be used for the determination of octanol-water coefficients (K_{OW}) for technical chlorflurenol-methyl and its separated components. The CCC can also be employed for the characterization and purification of the reaction products from synthesis of chlorflurenol-methyl.



I ($R_1 = R_2 = H$; $R_3 = CH_3$)	**Methyl 9-hydroxyfluorene-9-carboxylate ("Flurenol-methyl")**
II ($R_1 = Cl$; $R_2 = H$; $R_3 = CH_3$)	**Methyl 2-chloro-9-hydroxy-fluorene-9-carboxylate ("Chlorflurenol-methyl")**
III ($R_1 = R_2 = Cl$; $R_3 = CH_3$)	**Methyl 2,7-dichloro-9-hydroxy-fluorene-9-carboxylate ("Dichlorflurenol-methyl")**

Figure 1. Chemical structures for ingredients in technical chlorflurenol-methyl.

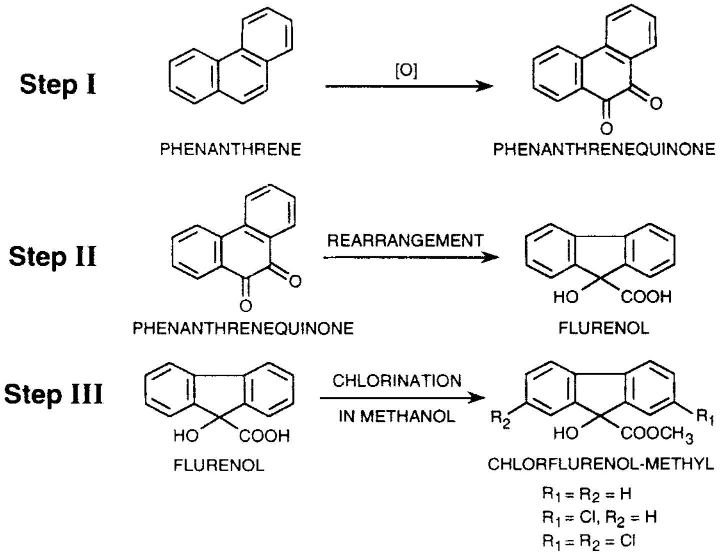


Figure 2. Synthetic scheme for chlorflurenol-methyl.

INTRODUCTION

Chlorflurenol-methyl is a common name assigned to a mixture of three components, viz., methyl 9-hydroxy-9-fluorenecarboxylate (I), methyl 2-chloro-9-hydroxy-9-fluorenecarboxylate (II) and methyl 2,7-dichloro-9-hydroxy-9-fluorene-carboxylate (III).

The mixture is also known as chlorflurecol-methyl, chlorflurenol methyl ester or chlorflurenol (Figure 1). The individual components are also referred to as flurenol-methyl (I), chlorflurenol-methyl (II) and dichlorflurenol-methyl (III).

This mixture (major component: chlorflurenol-methyl) is registered with the U.S. Environmental Protection Agency for use as a herbicide in controlling weeds in ornamental and turf grasses, and as a plant growth regulator for application to pineapples.[1]

The registered technical chlorflurenol-methyl is produced in three steps from phenanthrene which is used as a starting material. The synthesis involves oxidation of phenanthrene to phenanthrenequinone (see Step I in the flow-chart for the synthesis of chlorflurenol-methyl, Figure 2), which undergoes a rearrangement in the presence of alkali to give flurenol (Step II). Chlorination of flurenol in methanol gives technical chlorflurenol-methyl (Step III) which is a mixture containing methyl chlorofluorenol carboxylate (II), methyl dichlorofluorenol carboxylate (III) and methyl fluorenol carboxylate (I).

Previously, gas chromatography (GC) was used for the analysis of technical chlorflurenol-methyl,[2] which is the enforcement method approved by the EPA. This mixture can also be separated by high performance liquid chromatography (HPLC). The following conditions were used to separate the components from the mixture of chlorflurenol-methyl by HPLC (solvent: 30:70 acetonitrile-water; column: Fluofix; flow rate: 100 mL/min; detection: UV at 254 nm).[3] Proton nuclear magnetic resonance (^{1}H NMR) was also used for determining the composition of the components I, II and III in technical chlorflurenol.[3]

In this paper, we describe the separation of gram quantities of technical chlorflurenol-methyl by countercurrent chromatography (CCC), identification of the reaction products during synthesis (Figure 2), and the determination of octanol-water partition coefficients (K_{OW}) for the individual components (Table 1) separated by CCC.

Table 1

Octanol-Water Partition Coefficients for the Mixture and the Pure Components

Sample	Upper Phase (UP)	Lower Phase (LP)	K_{ow} = UP/LP	Log K_{ow}
Mixture (Lot #001-389)	0.111×10^3	0.042×3	881.0	2.94
Compound II (Chlorflurenol-methyl) (major component)	0.121×10^3	0.056×3	720.2	2.86
Compound III (Dichlorflurenol-methyl) (minor component)	0.220×10^3	0.050×3	146.7	2.17
Compound I (Flurenol-methyl) (minor component)	18×10^3	129×3	46.5	1.67

EXPERIMENTAL

Samples for CCC Separation and Analysis

The test sample (Fairmount Chemical Co., Newark, NJ; Lot Number: 001-389) was used for the large scale CCC separation and subsequent determination of octanol-water partition coefficients. It contains 12.98% flurenol-methyl (I), 71.26 % chlorflurenol-methyl (II), and 14.53 % methyl 2,7-dichloro-9-hydroxyfluorene-9-carboxylate (III).

The starting material and the synthetic intermediates were obtained from laboratory and pilot plant production batches following the synthetic scheme (Figure 2). These samples are the courtesy of Mr. C. Kwiatkowski of Roan Industries, Columbia, SC. The reference samples were obtained from Aldrich Chemical Co. (Milwaukee, WI).

CCC Instruments for Separation and Analysis

CCC instrument 1

It is a triplet coil centrifuge with 10 cm revolution.[4] It was used for the separation of gram quantities of technical chlorflurenol-methyl. The CCC column was prepared by using 2.6 mm ID (SW 10) PTFE tube wound in 7 layers of coil (J = 0.5 - 0.9). Three columns were connected in series to make a total capacity of 1600 mL. The suitable solvent system was found to be hexane: ethyl acetate: methanol: water (5:5:4:1) for preparative separation, using lower phase as the mobile phase. The partition coefficient (K = upper phase/lower phase) was 0.41 for this solvent system. For employing the upper phase as a mobile phase, the same solvent system [hexane: ethyl acetate: methanol: water (8:2:2:5)] with different proportions of the solvents in the mixture was used. For CCC separation, the test sample (3.0 g) was dissolved in a mixture of equal volumes of each phase (total volume: 85 mL). The CCC operating conditions include the following: centrifuge speed: 700 rpm (head to tail elution mode); flow rate: 5 mL/min; monitor/recorder: LKB Uvicord S at 254 nm, 2 abs. full scale; recorder chart speed: 1 cm/2 min; fractionation: 5 mL/min; temperature: 26.5°C.

For analysis and characterization of the intermediate products in the synthesis of chlorflurenol-methyl (Figure 2), a commercially available high-speed countercurrent chromatograph (CCC Instrument 2: Ito Multilayer Separator/Extractor, P.C. Inc., Potomac, MD) was used.

CCC instrument 2

In the Ito Multilayer Separator/Extractor (P.C. Inc.), the column was a multilayer coil of Tefzel tubing (1.6 mm ID and total capacity of 315 mL). The solvent system was hexane/ethanol/water (5:4:1) and the lower phase was used the mobile phase. The CCC operating conditions are the following: revolution: 800 rpm; flow-rate: 3 mL/min; pressure: 40 psi measured at the outlet of the pump. It is an analytical column and the sample sizes were 15 - 30 mg dissolved in the same solvent (concentration: 10 mg/L). Phenanthrenequinone (Step I product) and flurenol (Step II product) were analyzed under the above chromatographic conditions.

CCC Separation Procedure for Chlorflurenol-Methyl

Gram quantities of technical chlorflurenol-methyl (test material identified by Lot Number 001-389) were used for separation into components

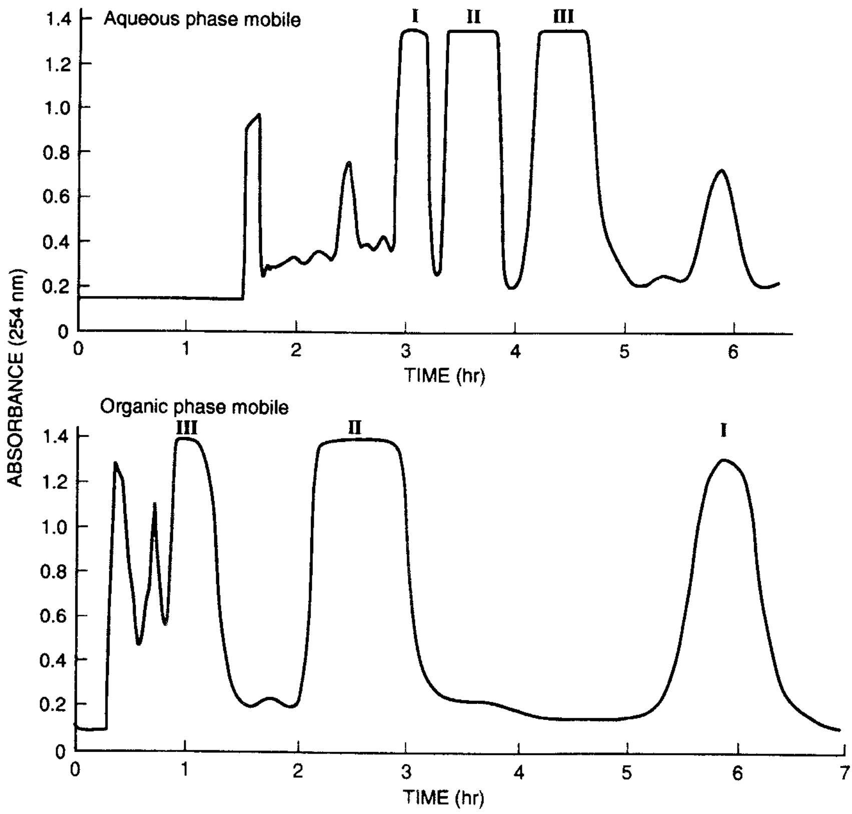


Figure 3. Separation of chlorflurenol-methyl on preparative-scale by countercurrent chromatography.

by instrument 1 which is a prototype developed by Ito and his coworkers.[3] The CCC analyses for the synthetic intermediates during the chlorflurenol production were also performed by using the two commercially available high-speed CCC instruments.

The general procedure involves the following: (1) the preparation of a two-phase solvent system in a separatory funnel at ambient temperature; (2) filling of the column first with the stationary phase (either aqueous or organic phase); (3) injection of the sample solution into the column; (4) keeping the column rotation speed at about 800 rpm; (5) elution of the column with the mobile phase at 5 mL/min.; (6) monitoring the absorbance at 254 nm; and (7) collection of the fractions from the column effluents for the isolation of the desired components from the test material (Figure 1) and the products from the synthetic program (Figure 2).

Analytical standards (compounds I, II and III) were prepared by separating the test sample (Lot Number: 001-389) on CCC instrument 1 (see Figure 3 for separation into individual components). These standards were used, in the present study, for the determination of octanol-water partition coefficient.

Procedure for Determination of Octanol-Water Partition Coefficients (K_{ow}) for Chlorflurenol-Methyl

Pure Chlorflurenol-methyl is sparingly soluble in water (solubility: about 15 ppm). The solubility of the mixture (test sample) may be higher (up to 100 ppm in water) because of the presence of other components.

Prepare a solution of the test material in octanol (10^{-2} to 10^{-3} M). Take 5 mL of the solution in 50 mL round-bottomed flask and add 25 mL of water. Stopper the flask and equilibrate the solution by shaking it overnight at ambient temperature (25°C). Separate the layers, after centrifugation (2000 rpm for 10 min), wash the upper phase with water and combine the lower phase with the water washings. Dilute both phases with methanol (upper phase requires 1000x dilution, whereas the lower phase needs 3x dilution). Determine the concentration of chlorflurenol in both phases by measuring its UV absorbance at 280 nm.

RESULTS

CCC Separations

The CCC profiles (see Experimental for chromatographic conditions of the CCC Instrument 1) for the separation of three components in technical chlorflurenol-methyl are shown in Figure 3. When aqueous phase was employed as a mobile phase (see the top CCC profile in Figure 2), the elution order is such that flurenol-methyl (I, a non-chlorinated product) elutes first followed by chlorflurenol-methyl (II which is the major component) and dichlorflurenol (III, which elutes last). The whole operation for CCC separation takes about 6 hours. When the phases are reversed (i.e., that the organic phase is used as a mobile phase), the elution order is reversed. In this solvent system [hexane: ethyl acetate: methanol: water (8:2:2:5)], the dichloro product (III) elutes first and the non-chlorinated flurenol (III) elutes last. The whole CCC operation takes about 7 hours for complete separation under these conditions (see the bottom CCC profile in Figure 3). The fractions corresponding to peaks I, II and III from chlorflurenol-methyl were collected and purified further to prepare analytical standards for determination of octanol-water partition coefficients.

The CCC profiles (see Experimental for chromatographic conditions of the CCC Instrument 2) for the intermediate products (step I and II products) in the synthesis of chlorflurenol-methyl (Figure 1) are shown in Figures 4 and 5.

Step I mixture, which is obtained by chromic acid oxidation of phenanthrene, was compared with the standard phenanthrenequinone (purity: 95%) obtained from Aldrich (Figure 4). The Step II product was compared with a standard, flurenol (9-hydroxyfluorene-9-carboxylic acid). The Step II product, which was identified as flurenol, was finally converted into chlorflurenol-method in Step III (Figure 5).

Octanol-Water Partition Coefficients

The results are summarized in Table 1. The octanol-water partition coefficient (K_{OW}) was calculated by using the formula:

K_{OW} = Upper Phase/Lower Phase (or UP/LP)

The results (Table 1) show that the octanol-water partition coefficient (K_{OW} for the mixture (which is the technical chlorflurenol) has a value of 262 whereas the pure chlorflurenol (Compound I) shows a K_{ow} value of 720.

DISCUSSION

Separation of the Components of Chlorflurenol-Methyl

A synthetic program was initiated for commercial production of chlorflurenol-methyl. Before scale-up operations, this substance was prepared on a laboratory scale. The same substance (Lot Number: 001-389) was used for developing the required health and safety data in support of the reregistration of chlorflurenol-methyl. Information on the octanol-water partition coefficients

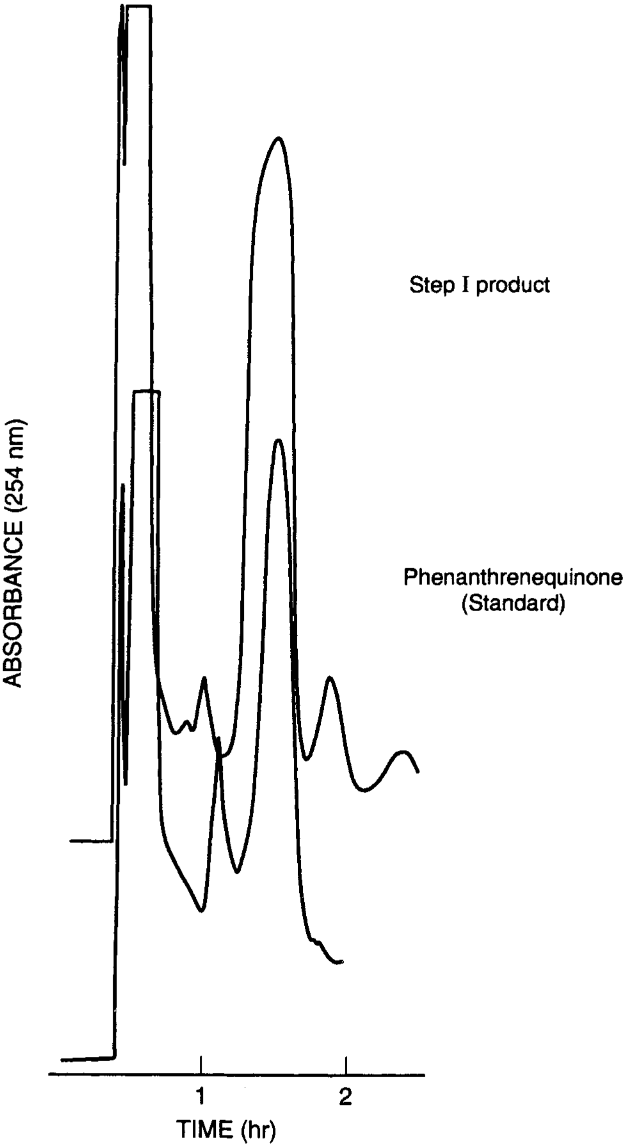


Figure 4. CCC profile for step I product containing phenanthrenequinone (major peak) (Top: Step I product and Bottom: Reference standard phenanthrenequinone from Aldrich Chemical Company).

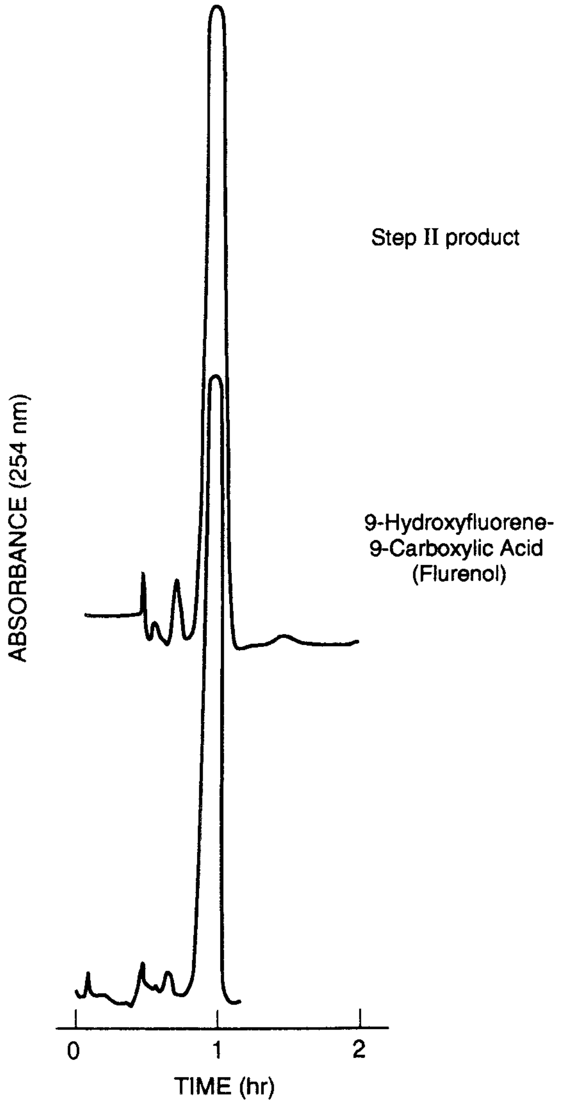


Figure 5. CCC profile for step II product containing flurenol (major peak). (Top: Step II product and Bottom: Reference standard 9-hydroxyfluorene-9-carboxylic acid from Aldrich Chemical Company).

(K_{ow}) of the components was required. In order to develop such information, pure samples of I, II and III were needed. In order to obtain them in pure form, preparative scale separation of chlorflurenol-methyl (Lot Number: 001-389) was undertaken with the use of the CCC Instrument 1 (see Experimental).

The CCC Instrument 1, in which three large columns (2.6 mm ID; column capacity: 1600 mL) were connected in series, was used for prep-scale separation. Several experiments were run in order to select a suitable solvent system, column size and CCC operating conditions before selecting hexane: ethyl acetate: methanol: water system. As shown in Figure 3, gram quantities (maximum 3 g/run) of chlorflurenol-methyl were used for successful separation into individual components I, II and III. After CCC separation, the fractions corresponding to the peaks I, II and III were collected and these fractions were further purified by CCC to remove any contaminants, if present in trace amounts. These components (I, II and III) were used for the determination of octanol-water partition coefficients (K_{ow}).

For the production of chlorflurenol, a good analytical method was needed to analyze (characterize) the products that are formed in different synthetic steps (Figure 1). The GC was found not to be useful for characterizing the Step II products (Figure 2) without converting them into volatile derivatives. Although the HPLC can be used for this type of analysis, the CCC was preferred because all the synthetic intermediates can be separated regardless of whether they are hydrophobic or hydrophilic in nature. The separated products were characterized by CCC comparison with those of the standards (Figures 4 and 5). The GC-MS was used to confirm the structures for these intermediate (reaction) products.

Octanol-Water Partition Coefficients

As stated above, the information on their partition coefficients in octanol-water (K_{ow}) is needed for the re-registration of chlorflurenol-methyl. The tendency of an organic chemical substance to bioconcentrate in living cells can be inferred from the value of the octanol/water partition coefficient (K_{OW}). Chemical substances with less than 10 will not significantly partition into, or tend to accumulate in living cells. Chemicals with greater than 10^6 will tend to accumulate. Chemical substances that exist in the environment at subtoxic levels may bioconcentrate to toxic levels once inside the organisms.

The octanol/water partition coefficient is useful in predicting whether a particular chemical has a tendency to bioaccumulate in aquatic organisms. If the chemical is insoluble in water, it is unlikely to be present in water and, hence, it is not available for bioaccumulation by the living organisms. Since chlorflurenol-methyl is practically insoluble in water (solubility: about 15 ppm), it is unlikely to be present in the biota and there is no likelihood that it has a potential for bioaccumulation in the marine organisms.

CONCLUSIONS

The CCC was successfully employed for the separation of gram quantities of technical chlorflurenol-methyl into three components which were isolated and purified. CCC was found to be very useful for characterization of the intermediate (synthetic) products during the manufacture of chlorflurenol-methyl. It can be used for the separation of impurities in the reaction product (containing phenanthrene-quinone and other impurities) after oxidation of technical phenanthrene (see Step I in Figure 1), which is hydrophobic, and conventional separation by HPLC or GC was difficult.

CCC was shown to be useful for determination of octanol-water partition coefficients (K_{ow}) of the three components present in the technical chlorflurenol-methyl.

ACKNOWLEDGMENT

The authors express their sincere appreciation to Mr. Charles Kwiatkowski of Roan Industries for providing chlorflurenol-methyl and other synthetic intermediate products used in this investigation.

REFERENCES

1. A portion of the material from this paper was presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, in Chicago, Illinois, March 4, 1996 (Abstract 171P).

2. E. Amadori, W. Heupt, "Chlorflurecol-Methyl," in **Analytical Methods for Pesticides and Plant Growth Regulators,** Gunter Zweig, ed., Volume X, Academic Press, New York, 1978, pp. 525-532.

3. N. B. Mandava, unpublished results.

4. Y. Ito, E. Kitazume, J. L. Slemp, "Improved High-Speed Countercurrent Chromatograph with Three Multilayer Coils Connected in Series. IV. Evaluation of Preparative Capability with Large Multilayer Coils," J. Chromatogr., **538**, 81-85 (1991).

Received March 20, 1997
Accepted June 2, 1997
Manuscript 4432