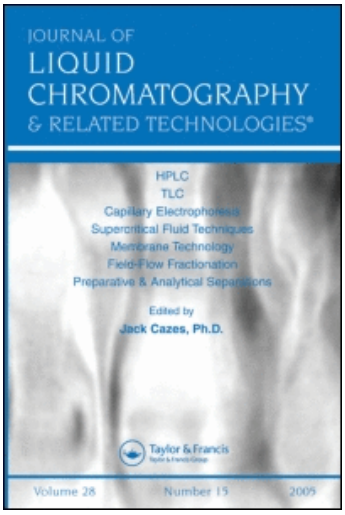

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Separation of S-Triazine Herbicides by Countercurrent Chromatography

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SEPARATION OF *S*-TRIAZINE HERBICIDES BY COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

Countercurrent chromatography (CCC) has been successfully applied for the separation of four chlorinated s-triazine derivatives, namely, simazine, atrazine, propazine, and trietazine, which are widely used as herbicides. Of several solvent systems investigated, n-hexane-ethyl acetate-methanol-water (8:2:5:5) gave the best range of the partition coefficient values because of the satisfactory solubility for all samples and therefore was used for the separation of s-triazines. Two types of countercurrent chromatographs, high speed CCC, and horizontal flow-through coil planet centrifuge, were used for s-triazines separation, and the identity of the separated fractions was unequivocally established by mass spectrometry. The potential practical application of CCC to herbicide analysis is discussed.

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INTRODUCTION

Herbicides based on the symmetrical triazine nucleus have been widely used for selective preemergence control of broadleaf weeds in croplands (1-3); and also used for nonselective and long-term weed control in noncrop situations (2-3). Some of them (e.g., simazine) are also used to control algae and submerged weeds in ponds, swimming pools and cooling towers (2,3). Most s-triazine herbicides have alkyl-substituted amino groups in positions 2 and 4, and either a chlorine atom or a methylthio group in position 6. The compounds containing a chlorine atom have common names terminating in -azine (e.g. atrazine), and those containing a methylthio group end in -etryn(e) (e.g. ametryne). Because of their extensive use in agriculture and because their physiology is well understood, we have chosen four chlorinated s-triazines for separation by countercurrent chromatography (CCC).

Several analytical methods, including gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC), have been used for analysis of chlorinated s-triazines. One of the approved analytical methods for all chlorinated s-triazines is GLC (4-6), although HPLC appears to be equally suitable for analysis and residue determination.

Countercurrent chromatography (CCC), based on the principles of countercurrent distribution and liquid chromatography, has been successfully applied for the separation of several types of both monomeric and polymeric compounds, including natural products (8, 9). We have previously reported the separation of a mixture of plant growth regulators such as indole auxins, gibberellins, cytokinins, and abscisic acid (ABA) by CCC (9) using a toroidal coil planet centrifuge. Recently, we have applied CCC for analysis of plant hormones and determined the ABA content in plant samples by a two-step CCC procedure (10). This paper deals with the application of CCC to the separation of a standard mixture of chlorinated s-triazine herbicides with a view to exploring the CCC potential for analysis and residue determination in the field of pesticides.

MATERIALS AND METHODS

Apparatus

In the present study, two types of CCC devices were evaluated for their performance. Both devices belong to a family of coil planet centrifuges (CPC) which produce a synchronous planetary motion identical to that in the toroidal coil planet centrifuge (9). The general operating conditions of these instruments are summarized in Table 1.

1) High Speed Countercurrent Chromatograph.

This CCC centrifuge performs fast and efficient separations in both analytical and semi-preparative scales, depending on the column. The design of the apparatus (Fig. 1) is also identical to that in the toroidal CPC except that the separation column consists of multiple layers of coiled PTFE tubing coaxially wound around a spool-shaped holder of 10 cm diameter. In order to facilitate the preparation of this multilayer coil column, the holder is made removable from the rotatory frame simply by loosening a pair of screws.

The unique feature of this CCC scheme is derived from an intriguing hydrodynamic motion of the two immiscible solvents in the multilayer coil. Under the synchronous planetary motion the two solvent phases

TABLE 1

General Operating Conditions for CCC Instruments in s-Triazine Herbicides Analysis

CCC Instrument	Flow Rate (ml/hr)	Revolutional Speed (rpm)
1. High speed counter-current chromatograph	150	800
2. Horizonal flow-through coil planet centrifuge	100	600

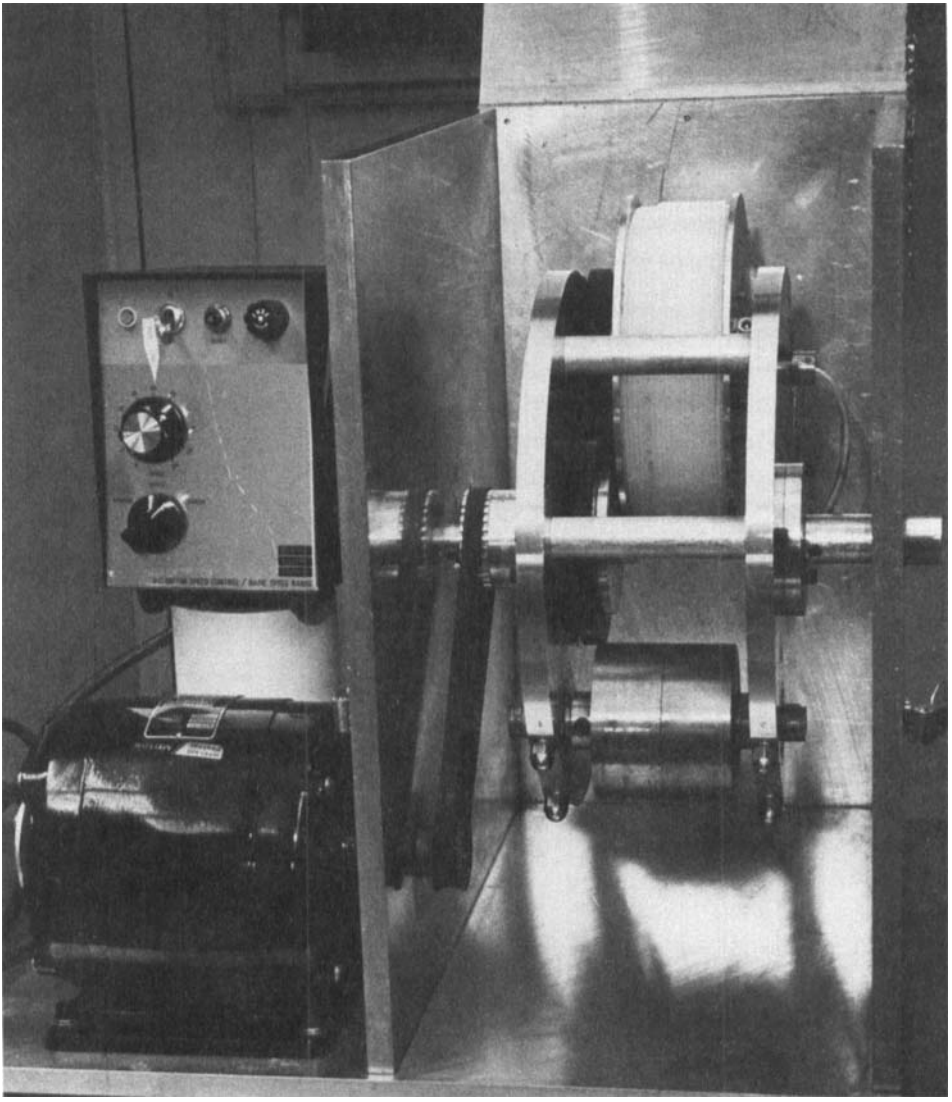

Figure 1. High Speed Countercurrent Chromatograph

are subjected to a rapid countercurrent flow along the length of the coil, with the upper phase traveling toward the internal head-end and the lower phase toward the external tail-end of the coil. This hydrodynamic motion establishes highly efficient partitioning of solutes with an excellent retention of the stationary phase under an unusually high flow rate of the mobile phase. Therefore, the method yields high peak resolution in a few hours of elution.

In the present study, separations are performed using a column of 130 m length, 1.6 mm I.D. and 285 ml capacity. The coil is first filled with the stationary phase, followed by sample injection through the sample port. Then, the mobile phase is pumped into the column while the apparatus is run at 800 rpm. Both the sample solution and the mobile phase are introduced through the internal head-end of the coil. If the mobile phase is the upper phase, the mobile phase and the sample solution should be introduced through the external tail-end of the coil. The flow rate applied to the column is 150 ml/hr. The eluate is continuously monitored with an LKB Uvicord S at 254 nm and fractionated with an LKB fraction collector.

2) Horizontal Flow-Through Coil Planet Centrifuge.

The second apparatus used in the present study is a table-top model of the horizontal flow-through coil planet centrifuge which performs efficient semi-preparative separations with a variety of conventional two-phase solvent systems (Fig. 2A). The above model is an improved version of the original bench-top model which is equipped with a 50 cm long column holder (11). Reduction of the length of the column holder to 20 cm in the present model enhances the mechanical strength and permits the applications of high revolutional speeds at 600 rpm. Consequently, the system yields high partition efficiency and provides the reliable stationary phase retention and the high flow rate of the mobile phase to allow speedy separations. The reduced column holder length was partially compensated by increasing the coil length with double layers of the coil mounted on each unit. As in the high speed CCC apparatus, the column holder is easily removed from the rotary frame by loosening a pair of screws on each bearing block.

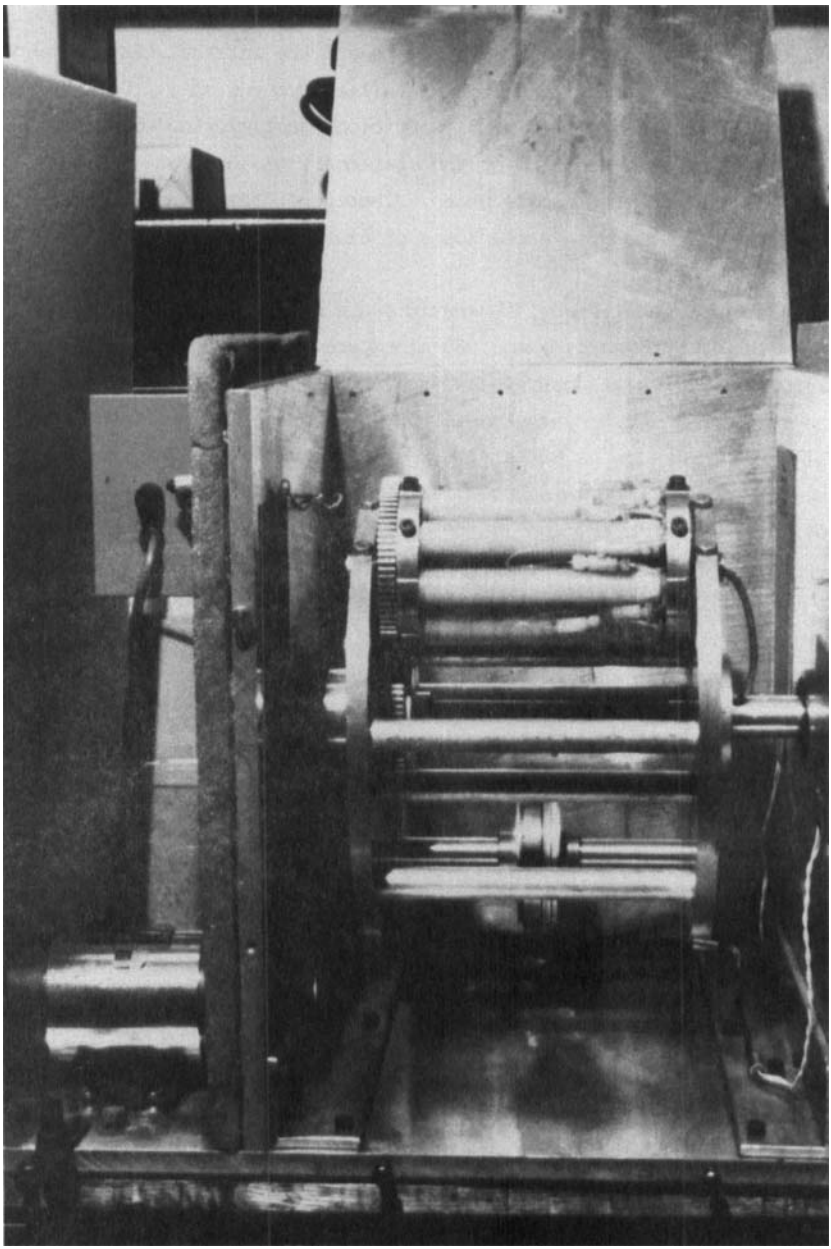

Figure 2. Horizontal Flow-Through Coil Planet Centrifuge.
A. Overview of the Table-Top Model.
B. Coil Assembly

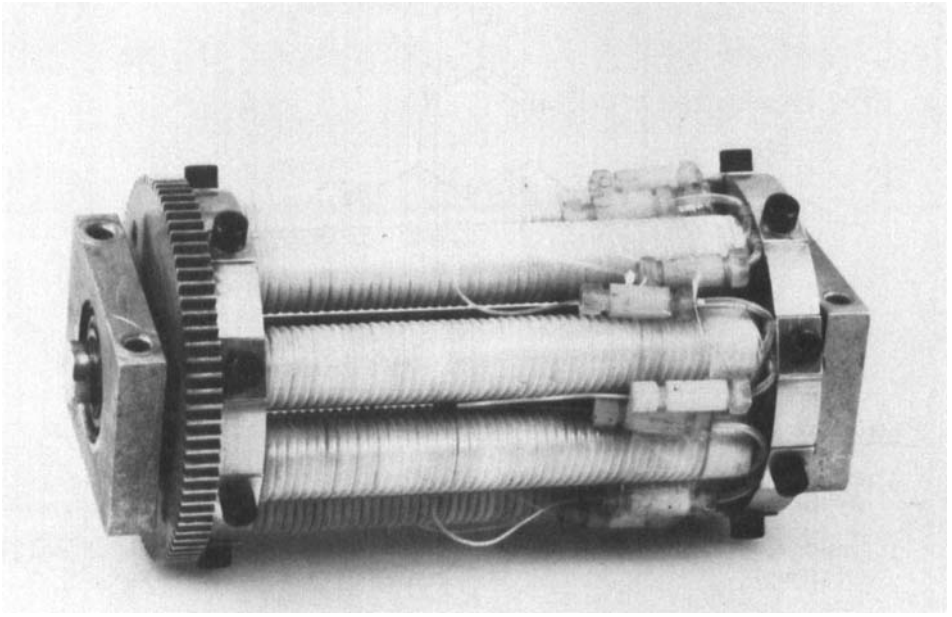

Figure 2B.

Each column unit was prepared from a 6 m. long, 1.6 mm I.D. PTFE tubing by winding it tightly onto a 14 cm long aluminum pipe core, making two coiled layers with about 100 helical turns. Eight one-column units were connected in series by the head-totail connections and symmetrically mounted around the column holder (Fig. 2B). The total column capacity measured approximately 100 ml.

Separation of the s-triazine herbicides was performed as follows. The column was first filled with the stationary (upper) phase and the sample solution was injected through the sample port. Then the column was rotated at 600 rpm while the lower phase was pumped into the head of the column at the rate of 100 ml per hour. The effluent from the outlet of the column was continously monitored with a Uvicord S at 254 nm and fractionated with an LKB fraction collector. After the first three peaks were eluted, the mobile phase was switched to the upper phase to quickly elute out the remaining peak from the column (Fig. 6).

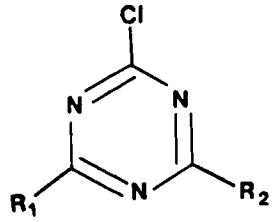

TABLE 2

Common and Chemical Names for Chlorinated s-Triazines

Structure	Common Name	Chemical Name
I (R_1=$NHMe_2$; R_2=NHEt)	Atrazine	6-Chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
II (R_1=R_2=$NHCHMe_2$)	Propazine	6-Chloro-N,N'-bis (1-methylethyl)-1,3,5-triazine-2,4-diamine
III (R_1=R_2=NHEt)	Simazine	6-Chloro-N,N'-dimethyl-1,3,5-triazine-2,4-diamine
IV (R_1=NHEt; R_2=NEt_2)	Trietazine	6-Chloro-N,N,N'-triethyl-1,3,5-triazine-2,4-diamine

Sources of s-Triazine Herbicides

All the test compounds were technical grade chemicals made by Ciba-Geigy Corp. They were used without further purification. The common names, approved by the American National Standards Institute (ANSI), were used for the chlorinated s-triazines whose chemical names are based on the chemical abstracts (CA) nomenclature (according to Ninth Chemical Index) as shown in Table 2.

Partition Coefficients

Partition coefficients were determined by adding a known amount of the chlorinated s-triazines in the two solvent systems used for each compound. After thoroughly mixing the compounds in the desired

solvent system, the two phases were separated and the absorbance measured at 260 nm. The ratio of absorbance values of the samples from two phases gave their partition coefficient (P.C.) in the respective solvent systems. A typical procedure for determining the P.C. of atrazine in n-hexane-ethyl acetate-methanol-water (8:2:5:5) is given here. To a mixture of 1.6 ml of n-hexane, 0.4 ml of ethyl acetate, and 1 ml of water was added 1 ml of atrazine solution in methanol (concentration: 1 mg/1 ml). The compound and the solvents were mixed vigorously and the two phases allowed to separate. A 0.5 ml volume of each phase was mixed with 3 ml of methanol. The absorbance was measured at 260 nm with a Zeiss UV-visible spectrophotometer, using a 1-cm light-path quartz cell, which gave readings of 0.294 and 0.208 for the upper (lighter) phase (C_u) and the lower (heavier) phase (C_l), respectively. Hence, the P.C. of atrazine is 1.41 (C_u/C_l) for the above solvent system.

Mass Spectrometry:

A Finnegan Model 4021 quadrupole mass spectrometer equipped with a Model 9610 gas chromatograph was used to analyze the chlorinated s-triazines. A Finnegan INCOS data system controls the automatic repetitive scanning, acquires and stores the data, and under the operator's instructions completes the data processing.

Samples were concentrated under a stream of nitrogen and introduced on the solid inlet probe. A portion (1 to 2 microliters) of the concentrate of each sample was transferred into a probe sample capillary tube and evaporated to dryness in a stream of air at room temperature. Fraction 11 from the first peak (Fig. 3) was permitted to evaporate before the heater was turned on. The other three fractions (17 from second the peak, 33 from the third peak and 73 from the last peak) were, in each case, heated (3 turns on heater potentiameter) to get more efficient evaporation within a shorter time interval. The last one (Fraction 73 from the fourth peak) was the most dilute, because of the greater width of the CCC peak, and was probably also the most volatile. Its concentration in the ion source was the lowest, but was still adequate for identification. The ion source temperature was 250°C. Electron energy was 50 eV.

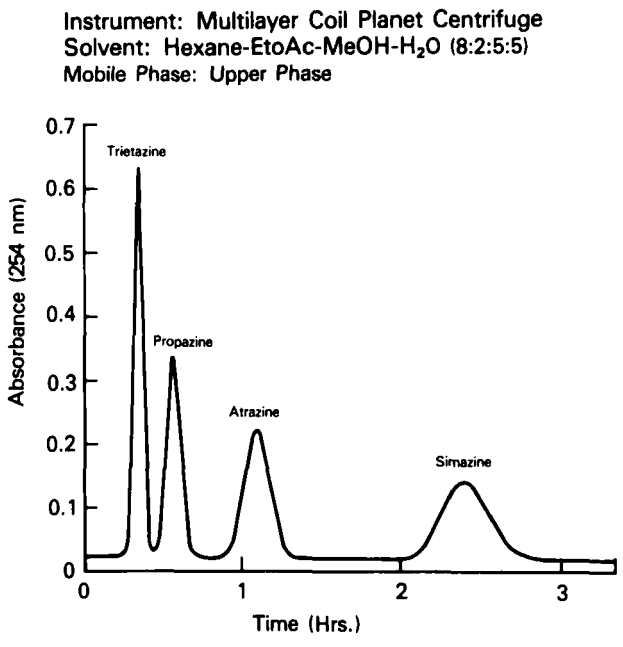

Figure 3. Separation of the Chlorinated s-Triazines by High Speed Countercurrent Chromatograph in Hexane-Ethyl Acetate-Methanol-Water (8:2:5:5). Upper Phase: Mobile.

RESULTS AND DISCUSSION

The choice of the two phase solvent systems was based on the partition coefficients (P.C.s) and the solubility of the samples. Several kinds of solvent systems were investigated. Among them, solvent systems composed of chloroform-methanol-water and chloroform-acetic acid-water at various volume ratios gave insufficient partition coefficient values, and most of the samples were partitioned into the lower non-aqueous phases. The two types of solvent system, n-hexane-methanol-water and n-hexane-ethyl acetate-methanol-water, yielded suitable ranges of partition coefficients as shown in Table 3. The n-hexane-ethyl acetate-methanol-water (8:2:5:5) gave the best range of the partition coefficient values and satisfactory solubility for all samples, and therefore was chosen for the present work. The P.C.s in this solvent system are shown in Table 4.

TABLE 3

Partition Coefficients of Chlorinated s-Triazines in Two Solvent Systems at Different Volume Ratios

	Solvent Composition	Volume Ratio	Partition Coefficient (C_u/C_l)*			
			Trietazine	Propazine	Atrazine	Simazine
1.	Hexane-Methanol-Water	2 : 2 : 0	0.51	0.38	0.30	0.28
		2 : 2 : 1	2.30	0.51	0.22	0.20
		2 : 1 : 1	4.57	1.16	0.43	0.16
		2 : 1 : 2	18.60	3.80	1.08	0.63
		2 : 0 : 2	108.00	14.70	3.09	2.00
2.	Hexane-Ethyl Acetate-Methanol-Water	5 : 5 : 5 : 5	9.02	6.10	3.71	2.02
		6 : 4 : 5 : 5	7.64	5.18	2.89	1.19
		7 : 3 : 5 : 5	6.95	4.41	2.16	0.92
		8 : 2 : 5 : 5	6.90	3.95	1.41	0.63
		9 : 1 : 5 : 5	5.41	2.37	1.10	0.35
		10 : 0 : 5 : 5	4.57	1.16	0.43	0.16

* C_u: Solute concentration in upper phase; C_l: Solute concentration in lower phase.

TABLE 4

Partition Coefficients of Chlorinated s-Triazines in Hexane-Ethyl Acetate-Methanol-Water (8:2:5:5)

Compound	Concentration (μg/ml)	Partition Coefficient*	
		C_u/C_l	C_l/C_u
Trietazine	250	6.90	0.14
Propazine	250	3.95	0.25
Atrazine	250	1.41	0.71
Simazine	250	0.63	1.60

* C_u: Solute concentration in upper phase; C_l: Solute concentration in lower phase.

With this range of P.C.s, the proper choice of the mobile phase should be made according to the retention capacity of the stationary phase inherent to the methods (12). High speed CCC, which produces high stationary phase retention, will yield rapid and efficient separations by using the upper phase as the mobile phase. On the other hand, the horizontal flow though coil planet centrifuge produces much lower retention of the stationary (lower) phase which amounts to substantially less than 50% of the total column capacity. In this case, the best results will be obtained by using the lower phase as the mobile phase. In this way, solutes are subjected to the partition process in the column for a longer period of time to yield higher peak resolution.

Because of the inherent differences in the P.C.s in this solvent system, one would expect the chlorinated s-triazines in a mixture to separate into individual components by CCC. If the lower phase is kept stationary on the column and the upper phase is used to elute the column, the partition coefficients (C_u/C_l) for chlorinated s-triazines (Table 4, column 3) serve as a useful guide for predicting the order of elution of those compounds in a mixture. When the phases are reversed, one must use column 4 (C_l/C_u) of Table 4. As the elution pattern follows the decreasing order of P.C.s, the separation of the mixture begins with the compound of highest P.C., followed by compounds with lower P.C.s.

In a typical experiment, a mixture (8 mg) containing atrazine, propazine, simazine, and trietazine (2 mg each), whose P.C.s vary from 0.63 to 6.90, was chromatographed on high speed CCC in hexane-ethyl acetate-methanol-water (8:2:5:5), keeping the lower (aqueous) phase stationary. These compounds eluted in the order that one would predict: trietazine (P.C.: 6.90) eluted first followed by propazine (P.C.: 3.95), atrazine (P.C.: 1.41) and simazine (P.C.: 0.63) with the upper (non-aqueous) phase as mobile phase (Fig. 3). The chromatographic separation of the mixture of chlorinated s-triazines was completed within 3 hours. The fractions were monitored with a UV monitor at 254 nm. The eluent was collected in 90 fractions (5.2 ml/fraction; flow rate: 2.6 ml/min) during the chromatographic separation of the mixture. The fractions corresponding to each UV peak were kept separate (1st peak: Fractions 10-11; 2nd peak: Fractions 15-19; 3rd peak: Fractions 30-35, and 4th peak: Fractions 66-79). Because of the homogenous nature of the fractions corresponding to peaks, only the middle fraction from each peak was analyzed by mass spectrometry. As expected, the mass spectrometric data confirmed trietazine under the first peak, propazine under the second peak, atrazine under the third peak, and simazine under the last peak (Figs. 4 and 5). This work unequivocally demonstrated that the partition coefficients give correct guidance during the separation of the mixture.

To show that the reversal of phase affects the order of separation, due to reversal of P.C.s (column 4, Table 4), the mixture (4 mg) containing the same chlorinated s-triazines (1 mg each) was chromatographed on a horizontal flow-through coil centrifuge, keeping the upper (non-aqueous) phase stationary. We have chosen to use the same solvent sytem but simply to reverse the phase, whereby the change in P.C.s (C_l/C_u) reverses the order of the elution pattern for these compounds (Fig. 6). In this way, simazine (P.C.: 1.60), which had the longest retention time in the above situation (Fig. 3) and had to be pumped out of the column with the stationary (upper) phase solvent, is now eluted with the shortest retention time (Fig. 6). Further, clean separations of simazine from propazine, and atrazine from propazine are made (Fig. 6), although the retention times are longer in this separation profile.

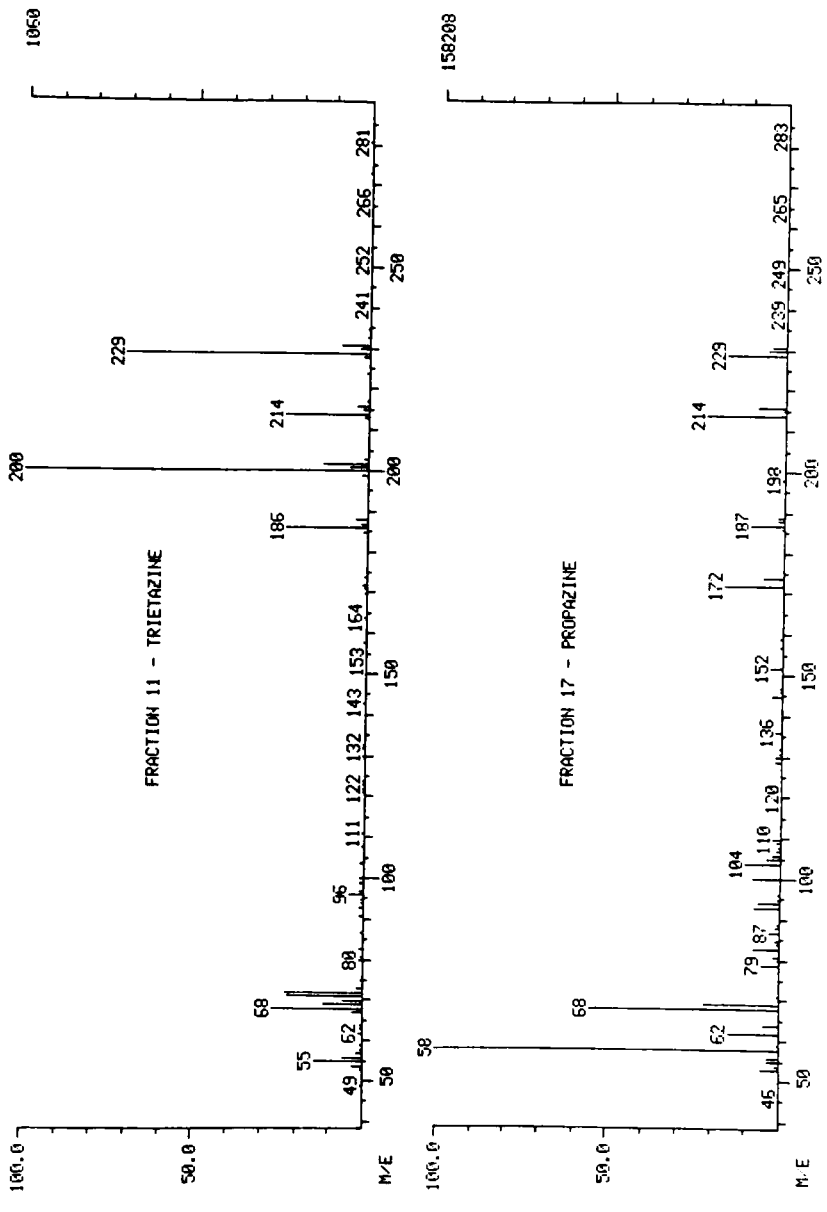

Figure 4. Mass Spectra of Fractions from CCC Separation (Figure 3). Top Spectrum: Fraction 11 (Trietazine). Bottom Spectrum: Fraction 17 (Propazine).

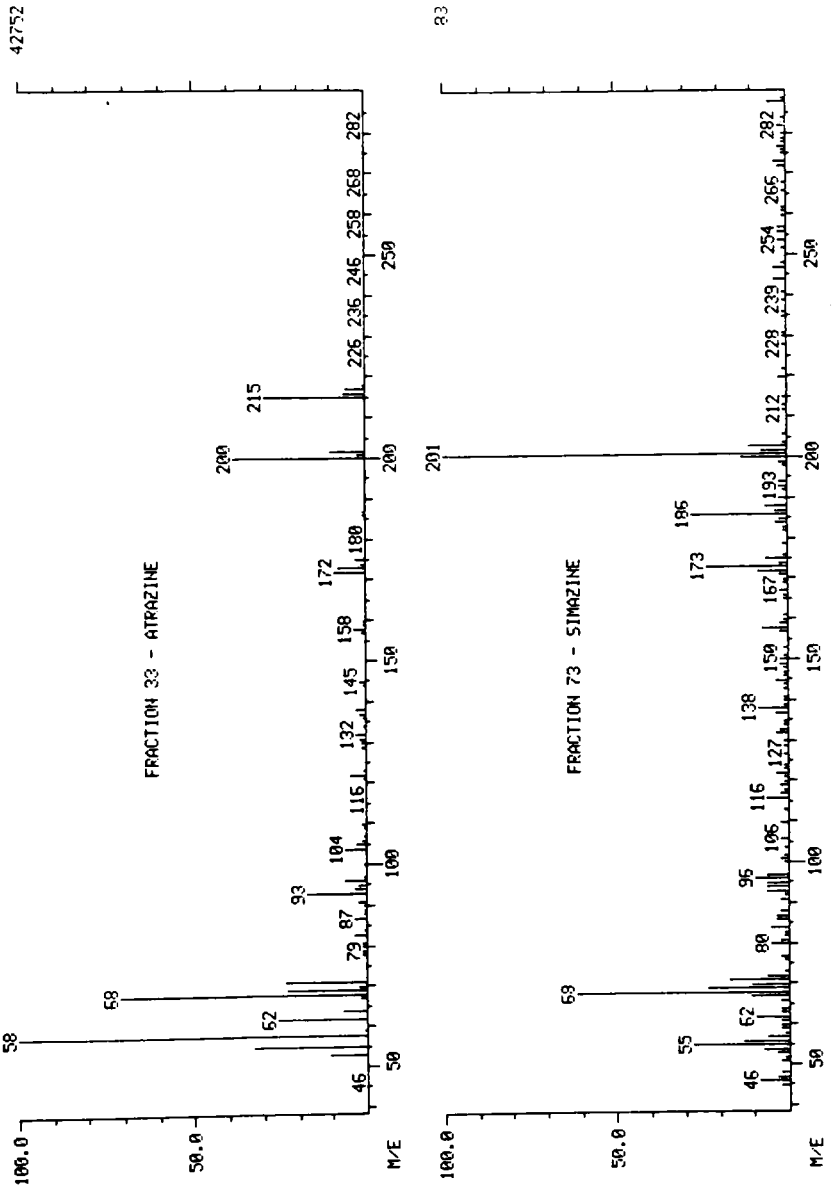

Figure 5. Mass Spectra of Fractions from CCC Separation (Figure 3). Top Spectrum: Fraction 33 (Atrazine). Bottom Spectrum: Fraction 73 (Simazine).

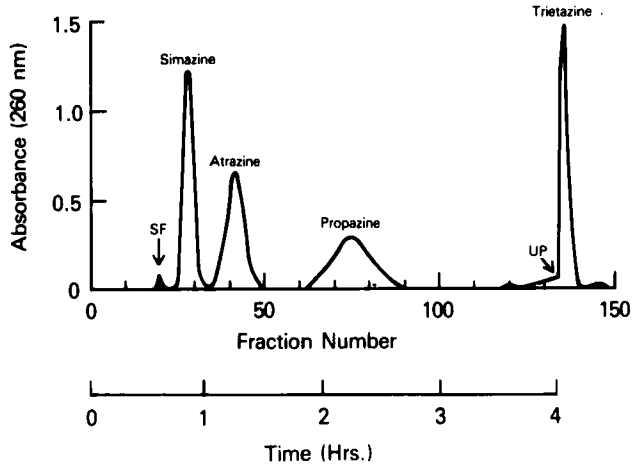

Figure 6. Separation of the Chlorinated s-Triazines by Horizontal Flow-Through Coil Planet Centrifuge in Hexane-Ethyl Acetate-Methanol-Water (8:2:5:5). Lower Phase: Mobile.

This approach demonstrated that:

1. The partition coefficient is either directly or inversely related to the retention times measured from the solvent front, and

2. Reversal of the choice for the mobile phase gives the reciprocal values of the P.C.s and therefore affects the retention times of the solute molecules.

As indicated previously (9), the approach has the following advantages:

1. Changing the phase from stationary to mobile is very easy to perform in CCC;

2. If a particular compound of interest takes a long time to elute from the CCC column, changing the phase reduces the retention time considerably;

3. For residue determination of chlorinated s-triazines from plant or soil samples, one could first separate the s-triazine(s) from other undesired compounds by a process where the s-triazine(s) can be collected first (with shorter or longer retention times) in a semi-preparative CCC mode and later, by reversal of the phase in CCC, one could obtain a pure compound on an analytical CCC column.

CONCLUSIONS

The work presented in this paper demonstrates that CCC appears to have great potential for the separation and analysis of s-triazine herbicides in an efficient manner. Neither expensive columns nor costly instruments are required in this type of separation. With the availability of different CCC instruments and columns, separation can be carried out on either an analytical or a preparative scale. When desired, gradient elution can be performed in a manner similar to HPLC. In retrospect, the method may have wide applicability for analysis of pesticide residues from water, soil, plant, and animal sources after conventional extraction methods give suitable fractions, each of which can be successfully analyzed following the procedures outlined above.

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DISCLAIMER

Mention of a trademark or proprietary product does not constitute a guaranty or warranty of the product by any Agency of the United States Government and does not imply its approval to the exclusion of other produts that may also be suitable.

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