Multimolecular Adsorption Chromatography for Purification of Gram Quantities of Pesticides

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A multimolecular adsorption chromatographic technique for purification of experimental pesticides or other organic liquids is described. One to 3 grams of 99+% pure product can be isolated from a 3- or 5-gram sample in 3 hours. Typical separations are illustrated, and the range of application is discussed.

Pure samples of experimental pesticides are difficult to obtain, if they cannot be distilled, recrystallized or synthesized in pure form. Molecular distillation or countercurrent extraction can be used to purify these compounds, but leave much to be desired in yield and convenience. Adsorption chromatography is normally limited to milligram quantities. Siegel and Mack (*2*) isolated 1 gram of decaborane employing a 4-foot column of Florisil (500 grams), but elution time was about 30 hours. Partition chromatography permits separation of larger samples per given amount of stationary phase, but involves considerable development time and choice of eluting solvents is limited.

The described procedure has proved versatile in purifying many types of experimental phosphate pesticides. It permits good separation of compounds which differ greatly in polarity and partial isolation of closely related compounds. The procedure involves chromatography of a 3- to 5-gram sample on only 50 grams of Florisil using a series of solvent mixtures having increasing polarity. Fifteen fractions are collected in 3 hours. The authors' experience is limited to compounds which adsorb on Florisil and which are eluted with 50% acetone in chloroform. Appropriate substitution of adsorbents and eluting solvents will extend this range.

A sample to adsorbent ratio of 1:10 is employed which is approximately 100 times greater than that used in conventional adsorption chromatography. Specific chromatographic procedures reported by Entel, Ruof, and Howard (*1*) used sample to adsorbent ratios of 1:10, but were tailored for individual cases. In the described procedure, Florisil with no free moisture was chosen as an adsorbent having medium adsorptive activity. The wide range in polarity of the eluting solvent mixtures gives the desired versatility. Multimolecular adsorption is the predominant mechanism of retention, since the theoretical capacity of the adsorbent is greatly exceeded with respect to monomolecular adsorption. This flooding of the adsorbent is usually expected to result in partial distribution of an impurity throughout the main component. However, it has been observed that there is a concentration of impurities at the leading and trailing edges of the main component, leaving the center portion highly purified. The degree of purity and separation achieved depends upon the sample.

Apparatus and Reagents

Column. Chromatographic column, 24 inches × 1 inch in O.D. with medium porosity, sintered-glass disk sealed in lower end.

Adsorbent. Florisil, 200- to 400-mesh, 1200° F. activation (Floridin Co., Tallahassee, Fla.); its free moisture is removed overnight in an 110° C. vacuum oven and it is stored in sealed jars.

Sample Preparation

Weigh 3 to 5 grams of sample into a 125-ml. Phillips beaker and dissolve in 50 ml. of hexane or the least polar solvent listed in Table I. Weakly adsorbed materials should be limited to 3 grams for optimum results. If sample mixtures contain components with very low solubility, it may be advantageous to chromatograph only the readily soluble portion.

Chromatographic Procedure

Prepare a slurry with 50 grams of Florisil in 150 ml. of hexane, rinse it into the chromatographic column, tap briefly to displace air bubbles, and apply 3 to 5 pounds, pressure to expel excess hexane. Add the 50 ml. of sample solution to the column, rinse with two 3-ml. portions of the same solvent, and force the sample into the packing. With two 3-ml. portions of the same solvent, rinse the column walls and force the solution into the packing. Place a tared, 125-ml. Phillips beaker into receiving position, add 75 ml. of hexane, and begin collecting fraction 1 at the rate of 6 to 8 ml. per minute (adjust pressure accordingly). Continue to add 75-ml. portions of increasingly polar solvents and collect as separate fractions (Table I). Add two 75-ml. portions of the last solvent. Due to the 94-ml. solvent holdup in the Florisil, there is a one-fraction lag between solvent addition and collection (a two-fraction lag for the last 19 ml.).

Table I. Solvent Mixtures and Elution Schedule

Solvent Composition, Volume %			Fraction No. (Collected)
Hexane[a]	Chloroform	Acetone	
(Sample solution solvent)			1
100	..	...	2
99	1	...	3
98	2	...	4
95	5	...	5
90	10	...	6
80	20	...	7
74.5	25	0.5	8
74	25	1	9
73	25	2	10
71	25	4	11
67	25	8	12
60	25	15	13
20	50	30	14
...	50	50	15

[a] Skellysolve B has been employed throughout.

Place the beakers in a 65° C. water bath and evaporate the solvent under a jet of filtered air. Remove the last traces of solvent by placing in a 65° C. vacuum oven for 30 minutes. Cool, weigh, and calculate the weight of each fraction.

The determination of fraction composition may be routine or rigorous, depending upon the specific chromatographic objectives and previous experience with the class of compounds involved. Fraction weights and refractive indices are convenient guides to distribution of sample components. Low recovery indicates the presence of volatile components, decomposition, or incomplete elution. Consecutive fractions which have a constant refractive index and/or other constant physical properties usually are a single compound of high purity. Suspected changes in fraction composition can be confirmed by a more specific analysis of key fractions.

Table II. Chromatographic Separation of Trithion and Analogs from Synthetic Mixture

Fraction Number	Weight, Mg.	Composition, %[a] Trithion	Oxygen[b] analog	Oxygen[c] analog sulfone
1	2	...	...	...
2	43	99+	...	...
3	332	99+	nil	nil
4	191	99+	nil	nil
5	128	99+	nil	nil
6	93	99+	nil	nil
7	307	6.2	93.8	nil
8	867	nil	99+	nil
9	1053	nil	99+	nil
10	756	nil	99+	nil
11	118	nil	86.2	13.8
12	125	...	nil	99+
13	247	...	nil	99+
14	5	...	nil	99+
15	2	...	...	...

[a] Fraction composition was determined by infrared and confirmed by ultraviolet spectroscopy. The word nil indicates <0.2%.
[b] *S*-(*p*-chlorophenylthio)methyl-*O*,*O*-diethyl phosphorothioate.
[c] *S*-(*p*-chlorophenylsulfonyl)methyl-*O*,*O*-diethyl phosphorothioate. These fractions were analyzed for Trithion and the oxygen analog only. They include small amounts of unidentified decomposition products.

Table III. Chromatographic Separation of Eptam from Synthetic Mixture

Fraction Number	Weight, Mg.	Composition, %[a] DEDTC[b]	Eptam	TPU[c]
1	554	67.9	32.1	<0.02
2	497	4.3	95.7	<0.02
3	176	0.3	99.6	0.1
4	115	0.2	99.7	0.1
5	108	0.1	99.8	0.1
6	124	0.1	99.8	0.1
7	149	0.1	99.8	0.1
8	358	0.04	99.9	0.1
9	574	<0.02	39.4	60.6
10	78	<0.02	0.54	99.5
11	11	...	...	...
12	nil	...	...	...
13	nil	...	...	...
14	nil	...	...	...
15	nil	...	...	...

[a] Fraction composition was determined by gas chromatography with analyses being presented as area %. [b] Diethyldithiolcarbonate. [c] Tetra-*n*-propylurea.

Decomposition during chromatography can be detected by comparison of physical, chemical, or biological properties before and after purification.

Chromatography of Synthetic Mixtures

To illustrate the degree of purification which had been achieved for typical sample mixtures, two synthetic samples were chromatographed. Table II summarizes chromatography of a 4.435-gram sample consisting of 18.11% Trithion or *S*-(*p*-chlorophenylthio)methyl-*O*,*O*-diethyl phosphorodithioate, 69.8% oxygen analog or *S*-(*p*-chlorophenylthio)methyl-*O*,*O*-diethyl phosphorothioate, and 12.09% oxygen analog sulfone or *S*-(*p*-chlorophenyl sulfonyl)methyl-*O*,*O*-diethyl phosphorothioate. Each component had been purified previously by the chromatographic procedure. Fraction composition indicated that separation was virtually complete and that only one mixed fraction existed between each component. Total sample recovery was only 96.4% due to some decomposition of the sulfone analog on Florisil.

Table III summarizes the results of a separation which was more difficult due to the relatively low adsorptivity of the three components. A 2.972-gram sample consisting of 69.06% Eptam (ethyl *N*,*N*-di-*n*-propylthiolcarbamate), 15.22% tetra-*n*-propylurea, and 15.72% diethyl dithiolcarbonate was chromatographed on Florisil containing no free water. Due to the relatively high vapor pressure of these components, the solvent was evaporated cautiously and the fractions were not placed in the vacuum oven. No decomposition was evident, but volatility loss reduced total recovery to 92.7%. This sample illustrates the chromatographic separation efficiency because of the sensitive, specific gas chromatographic method available for fraction analysis. Table III shows the effective isolation of 1 gram of 99.7+% Eptam, although only part of the Eptam was essentially free of the two impurities. In practice, Eptam is purified by fractional distillation under vacuum.

Discussion

The described procedure has been employed for several years and has proved invaluable in the preparation of 99.9% analytical reference samples of Trithion, Methyl Trithion, the five Trithion oxygen analogs, and many other experimental pesticides (structures are confidential).

Procedural modifications can be made when indicated by sample solubility, structural formula, or experience with similar compounds. Highest purification is effected for weakly adsorbed materials like Trithion when elution with hexane is continued for the first eight fractions. The possibilities of using adsorptivity tests, as described by Entel, Ruof, and Howard (*1*) have been explored, but a trial separation will yield more information for the time invested.

Limited experience has been gained in the successful purification of low melting solids such as the two sulfone analogs of Methyl Trithion where the sample remains an oil, except when highly purified. Samples with purity less than 50%, containing large amounts of impurities of similar structure, may have to be chromatographed twice to effect the desired purification. The procedure has been scaled up fivefold to chromatograph 25-gram samples by increasing the column cross-sectional area, adsorbent, and solvent, but maintaining the same depth of adsorbent. Florisil and Magnesol (*3*) have been employed as adsorbents, but other adsorbents may be more advantageous for some samples. Water, 3% or more, may be added to Florisil to deactivate it partially for use with strongly adsorbed or unstable materials. The choice of chloroform and acetone as eluting solvents was based upon the preparation of adsorption isotherms showing them to be only slightly adsorbed on Florisil and to function mainly as partitioning solvents rather than as displacement agents as in displacement chromatography.

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Literature Cited

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