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Short Communication

Multiple-development high-performance thin-layer chromatography of organochlorine pesticides

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ABSTRACT

Gradient multiple development on silica gel high-performance liquid chromatographic plates has been employed in this work to separate organochlorine pesticides. This method, even when performed with a limited number of development steps as in the present case, seems to give higher spot capacity and lower detection limits. Thus, wider screening possibilities for pesticide residue analysis can be expected to be introduced in the future employing multiple development gradients performed by automated techniques.

INTRODUCTION

The application of thin-layer chromatography (TLC) to the separation, identification and determination of organochlorine pesticides (OC) is well documented [1–6]. Among the various sorbents employed, silica gel and alumina [7] are the most popular. Plates with preadsorbent or concentration zones have been recommended because they allow sharper separations and higher sensitivity [8–10]. Many chromogenic reagents have been proposed [1,2,11–14]. For screening purposes silver nitrate, *o*-toluidine and 3,5,3′,5′-tetramethylbenzidine (TMB) seem to be the most suitable, the visual detection sensitivities ranging from 50 to 300 ng for many OC pesticides [13,15,16] and the lowest being obtained on silica gel plates with preadsorbent zones [16].

The coupling of a very efficient preconcentration technique with TLC separation allows the detection of OC pesticides at trace levels. Sherma [16] and Junk and Richard [17]. for instance, showed that using C_{18} solid-phase extraction it is possible, assuming 80% recovery, to achieve a detection limit of 0.06 ppb (10^9) for methoxychlor, lindane, endrin and DDT using a 1000-ml water sample. If lower detection limits are not

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required, modern TLC [18] may play a role in pesticide residue analysis for both screening and quantitative purposes [16].

In this paper, the gradient multiple development of some selected OC pesticides on high-performance (HP) TLC plates is described. In the multiple development process, the plate is repeatedly developed in the same direction with either the same or a different solvent, with drying of the solvent between runs. Each subsequent development moves the trailing edge of the zone closer to the front, resulting in narrower bands. This effect improves the spot capacity and sensitivity [19]. Gradient multiple development on silica gel plates is expected to enhance the visual detection limits, thus allowing improved screening analyses.

EXPERIMENTAL

Materials

Merck (Darmstadt, Germany) 5641 silica gel 60 HPTLC precoated plates, 10 × 20 cm, without fluorescence indicator, prewashed with dichloromethane–methanol (1:1) and then with pure methanol, were used. The solvents employed were *n*-heptane and dichloromethane of HPLC grade (Carlo Erba, Milan, Italy). The OC insecticides methoxychlor, dieldrin, endosulfan, lindane, *p,p'*-DDD, *p,p'*-DDE and aldrin were obtained from Supelco (Bellefonte, PA, U.S.A.) and used as received and dissolved in ethyl acetate to give 50–1000-ppm solutions.

Sample application

Standard solutions were applied to the plates as 10 mm wide bands with a Linomat IV (Camag, Muttenz, Switzerland) (1–3 μl; delivery speed 0.25 μl/s).

Chromatographic conditions

Ascending, one-dimensional, stepwise multiple development in a twin chamber (Camag), without chamber saturation, was applied. The temperature was 21–23°C and the relative humidity 60–65%.

The mobile phases were *n*-heptane–dichloromethane in the following proportions, with the distances run in each development step as indicated: (1) 55:45, 14 mm; (2) 65:35, 28 mm; (3) 75:25, 42 mm; (4) 85:15, 56 mm; (5) 95:5, 70 mm. After each development the plate was dried in a stream of nitrogen for 2 min. The time for a complete run was about 50 min.

Detection

The developed plates were dipped either in 0.5% ethanolic solutions of silver nitrate containing 5% concentrated ammonia solution or 0.1% acetone solutions of 3,5,3',5'-tetramethylbenzidine (TMB).

The dried plates were irradiated with short-wavelength UV light for 30 min. The pesticides were detected on the layer as black-brown spots on a white background with silver nitrate and yellow-brown spots on a clear background with TMB. The spots obtained in both instances remain stable for several days if the dipped layer is kept in a refrigerator.

The derivatized layers were scanned with a Camag Scanner II equipped with a Merck–Hitachi chromato-integrator. The reflectance was measured at 550 nm.

RESULTS

The choice of the solvent for multiple development depends on the width of the polarity range of the sample components. Samples with wide polarity ranges require so-called "universal gradients" that are useful for general purposes [18]. They consist of solvent mixtures starting with a very polar and ending with a very non-polar solvent, *e.g.*, methanol–dichloromethane–*n*-hexane.

In the present instance, owing to the relatively narrow polarity range of the OC insecticide mixture, a two-component solvent, one of medium polarity and the other of low polarity, proved to be appropriate. Dichloromethane was chosen from other possible medium-polarity solvents because, in addition to showing good selectivity, it could be used in a relatively wide composition range (from 45% to 5%) in mixtures with *n*-heptane. *n*-Heptane was chosen as a non-polar solvent because it showed a better mobility (20%) than *n*-hexane.

Some isocratic runs were carried out as preliminary steps for the gradient set-up. The results of these experiments are shown in Fig. 1 as plots of R_M *versus* volume fraction (φ) of dichloromethane for the eight OC insecticides. These isocratic data and those with multiple development were obtained under unsaturated conditions; better separations took place under these conditions than under saturated conditions, as observed previously by Gocan and Marutoiu [20].

It is noteworthy that in the experimental conditions adopted (relative humidity 60–65% and no preadsorption of the solvent vapour on the layer), a linear relationship between R_M and volume fraction, φ, of dichloromethane was obtained, as shown in Fig. 1. In contrast, a linear relationship between R_M and log φ, generally expected in normal-phase liquid chromatography, was found for the eight insecticides if the layers were allowed to preequilibrate with the solvent vapour before the development.

From the plots in Fig. 1, it is apparent that solvents with $\varphi \geqslant 0.40$ should be used

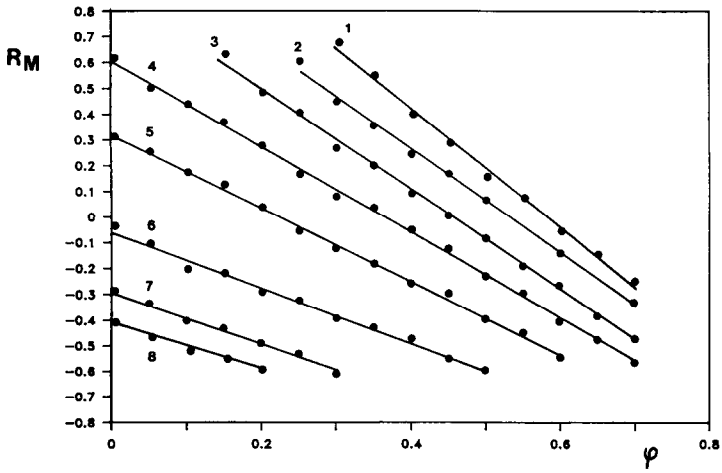

Fig. 1. Plot of R_M *versus* volume fraction of dichloromethane (φ) on HPTLC silica gel layers. OC insecticides as listed in Table I.

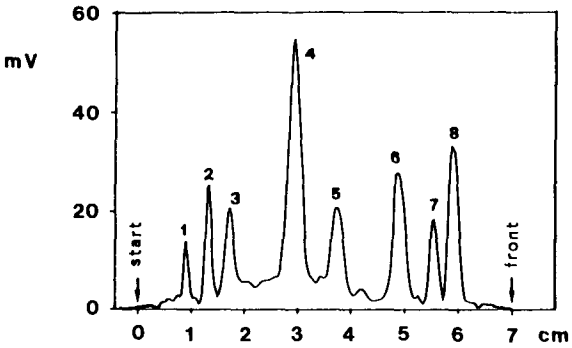

Fig. 2. HPTLC of OC insecticides. Densitogram of HPTLC plate after multiple development and derivatization with ethanolic silver nitrate, according to Sherma [16]. OC insecticides as in Table I. Amount of insecticide applied from 50 ng to 1 μg per spot.

as a first step in the gradient development in order to move appreciably methoxychlor, the most polar of the insecticides. On the other hand, a solvent of low polarity must be used in the last steps of the gradient in order to separate aldrin and *p,p'*-DDE. This separation is only possible in chromatographic zones not too close to the solvent front [21].

A few experiments were sufficient to devise the five-step optimized gradient described under Experimental. Fig. 2 shows the densitogram of the eight standards, well separated into narrow bands with a homogeneous distribution in the chromatographic space. An appreciable band reconcentration effect may be observed here and in Table I, where the limits of visual detection of the OC are reported.

In conclusion, multiple development on silica gel HPTLC plates increases the screening possibilities in pesticides residue analysis. Further, the results can be significantly improved by using gradients with a larger number of development steps as performed by automated techniques [6,18].

TABLE I

VISUAL DETECTION LIMITS OF ORGANOCHLORINE PESTICIDES (ng/mm^2)

No.	Compound	Silver nitrate + UV	TMB + UV
1	Methoxychlor	1	10
2	Dieldrin	15	15
3	α-Endosulfan	50	2
4	Lindane	5	5
5	*p,p'*-DDD	15	25
6	*p,p'*-DDT	1	3
7	*p,p'*-DDE	5	10
8	Aldrin	10	25

REFERENCES

1 J. Sherma, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. VII, Academic Press, New York, 1973, p. 3.
2 J. Sherma, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. XI, Academic Press, New York, 1980, p. 79.
3 V. N. Mallet, in J. Harvey and G. Zweig (Editors), *Pesticide Analytical Methodology (ACS Symposium Series*, No. 136). American Chemical Society, Washington, DC, 1980, p. 137.
4 M. E. Getz, *Paper and Thin-Layer Chromatography of Environmental Toxicants*, Heyden, London, 1980.
5 J. Sherma, *J. Liq. Chromatogr.*, 5 (1982) 1013.
6 J. Sherma, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. XIV, Academic Press, New York, 1986, p. 1.
7 J. M. Follweiler and J. Sherma, *Handbook of Chromatography —Pesticides*, Vol. 1, CRC Press, Boca Raton, FL, 1984.
8 J. Sherma, *Am. Lab. (Fairfield, Conn.)*, 10 (1980) 105.
9 J. C. Touchstone and S. S. Levin, *J. Liq. Chromatogr.*, 3 (1980) 1853.
10 H. E. Hauck and E. Amadori, in J. Harvey and G. Zweig (Editors), *Pesticide Analytical Methodology (ACS Symposium Series*, No. 136), American Chemical Society, Washington, DC, 1980, p. 159.
11 V. M. Adamovic, *J. Chromatogr.*, 23 (1966) 274.
12 M. Beroza, J. Sherma and J. F. Thompson, *Analysis of Pesticide Residues in Human and Environmental Samples*, U.S. Environmental Protection Agency, Triangle Park, 1977.
13 J. Makhubalo, A. Mainga and A. Phiri, *J. Chromatogr.*, 284 (1984) 518.
14 J. Sherma, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. XIV, Academic Press, New York, 1986, p. 14.
15 A. Amrus, E. Hargital, G. Karoly, A. Fulop and J. Lantos, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 743.
16 J. Sherma, *J. Liq. Chromatogr.*, 11 (1988) 2121.
17 G. A. Junk and J. J. Richard, *Anal. Chem.*, 60 (1988) 451.
18 D. E. Jaenchen, *J. Liq. Chromatogr.*, 11 (1988) 1941.
19 C. F. Poole and S. K. Poole, *Anal. Chem.*, 61 (1989) 1257A.
20 S. Gocan and C. Marutoiu, *Rev. Chim. (Bucharest)*, 32 (1981) 166.
21 F. Geiss, *Fundamentals of Thin-Layer Chromatography (Planar Chromatography)*, Hüthig, Heidelberg, 1987, p. 321.