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Note

Fluorescence of pesticides on silica gel thin-layer plates by use of a gaseous electrical discharge technique

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It has been found that the action of heat[1], heat in the presence of nitrogen[2,3] or a gaseous electrical discharge in the presence of nitrogen followed by heat[4] has the effect of selectively enhancing, and in some cases inducing, fluorescence in a wide range of organic compounds on thin-layer plates, with the resultant lowering of detection limits. The only requirement for a compound to fluoresce is that it should possess some degree of aromaticity.

The number of organic pesticides that fluoresce naturally is restricted but many are sufficiently aromatic to be converted into fluorescent species in the ways mentioned. The heating at between 200 and 225° for 30 min of 14 organophosphorus pesticides produced fluorescence or fluorescence enhancement in eight of these[1]. The visual detection limits ranged from 100 ng to 1 ng. In a study of the action of heat on compounds in the presence of nitrogen, methoxychlor was detected at the sub-nanogram level[3]. With the technique employing an electrical discharge[4], lindane and dieldrin were detectable at the 300–600 ng level.

Apart from the experiments referred to above, pesticides in general have not been studied. This note records the effects of the electrical discharge technique in selectively lowering the detection limits of a wide range of pesticides.

EXPERIMENTAL

A similar apparatus to that used previously[4] was employed (Fig. 1). The thin-layer plate is introduced and the system sealed. The high-frequency coil is switched on and the vacuum lowered until the glow discharge reaches its maximum intensity. The discharge is maintained for a predetermined time, then the vacuum is broken and the plate removed. Heating of the plate in an oven is followed by exposure to UV radiation.

Previous experiments have shown that thin-layer plates incorporating an organic binder give a significant background fluorescence[2,4]. It was, however, commented that Merck high-performance silica gel plates gave very compact spots and comparable sensitivities to plates without the organic binder. Merck HPTLC plates (E. Merck, Darmstadt, G.F.R.) were used in the experiment reported here.

Preliminary work showed that the optimum conditions for the electrical discharge experiment were as follows: 4 min in the electrical discharge chamber; 8 min in a forced draught oven at 130°; and 12 min under 350-nm UV radiation.

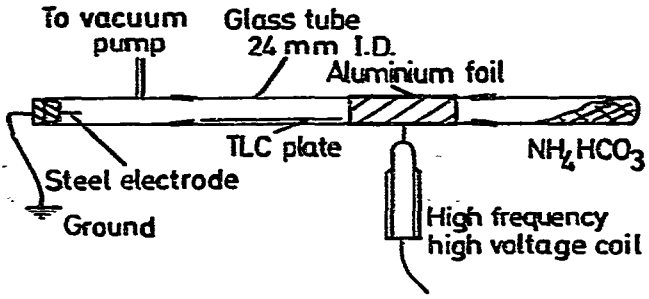

Fig. 1. Schematic diagram of the electrical discharge chamber. A Tesla high-frequency high-voltage coil is used, together with an Edwards Speedivac 2 vacuum pump. The ammonium hydrogen carbonate is the source of nitrogen.

Pesticide solutions (1 μg/μl) were made up in appropriate solvents and spotted as follows: 1 μl and 0.5 μl with a 1-μl Drummond micropipette (Drummond, Broomall, Pa., U.S.A.); 200 nl at concentrations of 1 μg/μl and 0.25 μg/μl, both with a 200-nl platinum-tipped Camag pipette (Camag, Muttenz, Switzerland).

RESULTS

The levels at which the various pesticides were detected are shown in Table I. It can be seen that heating for 8 min followed by exposure to UV radiation for 12 min did not have any effect on the fluorescence. In contrast, the electrical discharge technique lowered the detection limits in the case of six pesticides, producing fluorescence in four that do not fluoresce naturally.

TABLE I

DETECTION LIMITS OF PESTICIDES ON HPTLC SILICA GEL PLATES

ED = Electrical discharge; UV = ultraviolet radiation (350 nm); 1, 0.5, 0.2 and 0.05 = amount spotted in μg; + = detectable visually; − = not detected.

Pesticide	*UV only*				*Heat plus UV*				*ED plus N_2 plus heat plus UV*			
	1	*0.5*	*0.2*	*0.05*	*1*	*0.5*	*0.2*	*0.05*	*1*	*0.5*	*0.2*	*0.05*
Diuron	+	+	−	−	+	+	−	−	+	+	+	−
Atrazine	+	+	+	−	+	+	+	−	+	+	−	−
Picloram	+	+	−	−	+	+	−	−	+	+	−	−
γ-BHC	−	−	−	−	−	−	−	−	−	−	−	−
Endosulfan	−	−	−	−	−	−	−	−	−	−	−	−
p,p'-DDT	−	−	−	−	−	−	−	−	+	+	−	−
PCP	−	−	−	−	−	−	−	−	+	+	−	−
Monocrotophos	+	+	−	−	+	+	−	−	+	+	−	−
Dimethoate	+	+	−	−	+	+	−	−	+	+	−	−
Fenitrothion	+	+	−	−	+	+	−	−	+	+	+	−
Diazinon	+	+	−	−	+	+	−	−	+	+	−	−
Propoxur	−	−	−	−	−	−	−	−	+	+	−	−
Methyl gusathion	+	+	+	+	+	+	+	+	+	+	+	+
Perthane	−	−	−	−	−	−	−	−	+	+	−	−

In Table II the detection limits of other techniques are compared with those obtained in this study. In each case the limits of the other techniques are lower by from one to many orders of magnitude.

TABLE II

DETECTION LIMITS OF PESTICIDES BY VARIOUS TECHNIQUES ON TLC PLATES

Values in ng, except as indicated.

Pesticide	*This study*	*Various techniques*
Diuron	200– 50	1 ppm*
Atrazine	500–200	50**
Picloram	500–200	1 ppm*
γ-BHC	—	1***
Endosulfan	—	50***
p,p'-DDT	500–200	2***
PCP	500–200	100***
Monocrotophos	500–200	?
Dimethoate	500–200	200§
Fenitrothion	200– 50	?
Diazinon	500–200	50***; 5§
Propoxur	500–200	?
Methyl gusathion	<50	?
Perthane	500–200	20***

* UV (ref. 9).

** Chlorination[8].

*** Silver nitrate[5].

§ Enzyme inhibition[6,7].

An experiment carried out using fluorescence quenching gave the results recorded in Table III. 200 nl was spotted on HPTLC plates containing a fluorescent indicator (254 nm) and a chromatogram obtained using a Vitatron TLD 100 densitometer (Vitatron, Dieren, The Netherlands) at the following wavelengths: excitation, 280 nm; emission, 525 nm. The inconsistency of the results compared to those in Table I is due to the fact that each pesticide has its own combinations of excitation and emission wavelengths[1]. A lack of suitable filters did not allow individual pesticides to be quantitated precisely. The results are interesting, however, in that *p,p'*-DDT and PCP gave responses whereas they do not appear in Table I. The reverse situation occurs with monocrotophos and dimethoate.

The use of appropriate filters in the quenching and nonquenching mode should therefore extend the usefulness of the heating and electrical discharge techniques.

CONCLUSIONS

The attractiveness of the electrical discharge technique in enhancing and inducing fluorescence lies in its simplicity and application to a wide range of pesticides, and its uniformity compared with methods employing various spray reagents. The use of a single spray application (a strong acid or base) prior to heating has been found to improve detection limits in some cases[1,10].

While the detection limits are not yet equal to or better than those obtained

TABLE III

QUANTITATION USING FLUORESCENCE QUENCHING WITH 200 ng OF PESTICIDE SPOTTED ON TLC PLATES

Values represent chromatogram peak areas, the averages from duplicate determinations. The average relative error is ± 10.9% (S.D. = 8.3). Wavelengths used: excitation, 280 nm; emission, 525 nm. Other symbols as for Table I.

Pesticide	*UV only*	*Heat plus UV*	*ED plus N_2 plus heat plus UV*
Diuron	40	37	51
Atrazine	26	33	48
Picloram	72	67	48
γ-BHC	—	—	—
Endosulfan	—	—	—
p,p'-DDT	—	9	11
PCP	15	13	7
Monocrotophos	—	—	—
Dimethoate	—	—	—
Fenitrothion	27	25	—
Diazinon	—	—	—
Propoxur	15	43	34
Methyl gusathion	67	57	33
Perthane	7	36	33

with other techniques, the future development of high-performance silica gel and alumina-coated plates without a binder should improve the limits. It has been reported that alumina-coated plates are superior to those coated with silica gel[2]. With more suitable thin-layer plates it is felt that the electrical discharge technique will find applications in future experimentation. One probable requirement in practice will be that the sample extract loaded on to the plate, e.g., in pesticide residue work, should be very clean since the method is non-specific.

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