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THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION OF THE ACTIVE INGREDIENTS OF MIXED HERBICIDE FORMULATIONS

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SUMMARY

Applications of thin-layer chromatography to the identification of the components of herbicide mixtures are discussed. The compounds concerned include phenoxyalkanoic acids, triazines, carbamates and substituted ureas. Various separatory systems and visualization agents are described.

INTRODUCTION

The applications of thin-layer chromatography to pesticide residue analysis have been many and varied[1]. The procedures are ideal for confirmation of identity[2], for combined separation and clean-up stages[3], and for semi-quantitative evaluation based on spot-size or density measurements[4,5]. However, the usefulness of thin-layer chromatography in the examination of pesticide formulations or technical grade materials[6,7] appears to have been largely overlooked, though there are several possible areas in which it can be applied. Cross-contamination of formulated products has been shown to be of importance[8] and thin-layer chromatography offers a rapid and simple test procedure for such inadvertent occurrences. The presence, or absence, of allied by-products, isomers or extraneous materials in technical grade pesticides can often be checked readily, and a semi-quantitative estimation of such materials may also be possible by suitable examination of the developed chromatoplate.

There appears to be a growing tendency to market pesticide formulations containing two, three or even more active ingredients; this is particularly noticeable in regard to herbicide formulations. Table I includes the mixtures of this nature that have received approval in the United Kingdom under the Agricultural Chemicals Approval Scheme[9]; about thirty compounds are included in such mixtures in various combinations. Methods advocated for the quantitative analysis of herbicides and their formulated products are often non-specific in nature. Thus, the chlorophenoxyalkanoic acid compounds are usually determined by an acid-base titration method[10] and similarly the carbamate herbicides propham and chlorpropham are determined

by titration of the amine that is liberated on hydrolysis[11]. It is therefore essential to have some means of identifying the components of herbicide mixtures, preferably in such a way as to be able to establish quantitatively the ratio of the compounds present. Gas–liquid chromatographic methods may be applicable to the identification of many compounds but quantitative aspects are not as satisfactory as the more traditional methods[12]. Moreover, the use of gas–liquid chromatography often involves conversion to volatile derivatives, such as esters of the chlorophenoxyalkanoic acid herbicides, and the apparatus required is costly and requires careful handling. The simpler, quicker procedures of thin-layer chromatography therefore promised to be very useful for checking the nature of the active ingredients present in herbicide mixtures and the present note describes some separatory systems suitable for this purpose. These methods have been developed on behalf of the Herbicides Sub-committee of the U.K. Pesticides Analysis Advisory Committee.

EXPERIMENTAL

A number of herbicidal compounds that occur in mixed formulations have been studied with a view to establishing suitable conditions for their separation, identification and estimation by means of thin-layer chromatography. The compounds concerned fall into two broad classes: (a) the acidic compounds, and (b) the nitrogenous herbicides including carbamates, substituted ureas, triazines etc.

TABLE I

COMPONENTS OF SOME MIXED HERBICIDE FORMULATIONS[9]

(a) Two-component systems

Compound	*Second ingredients*
MCPA	mecoprop; MCPB; dichlorprop; 2,4-DB; benazolin; dicamba; 2,3,6-TBA; bromoxynil
Mecoprop	MCPA; 2,4-D; dichlorprop; fenoprop; dicamba; 2,3,6-TBA; ioxynil
MCPB	MCPA; benazolin
2,4-D	mecoprop; 2,4-DB; dichlorprop; 2,4,5-T; monuron; bromacil
Dichlorprop	MCPA; mecoprop; 2,4-D; picloram
2,4-DB	MCPA; 2,4-D; benazolin
Chlorpropham	propham; diuron; fenuron; linuron
Monolinuron	linuron; dinoseb
Simazine	prometryne, methoprotryne
Paraquat	diquat

(b) Multi-component systems

MCPA + mecoprop + dicamba
MCPA + MCPB + benazolin
MCPA + 2,4-D + 2,4-DB
MCPA + 2,4-DB + benazolin
MCPA + benazolin + dicamba
MCPA + atrazine + 2,3,6-TBA
MCPA + dichlorprop + ioxynil
Propham + endothal + medinoterb acetate
Chlorbufam + dimexan + cycluron
Chlorpropham + propham + fenuron
Atrazine + MCPA + 2,3,6-TBA
MCPA + mecoprop + dicamba + 2,3,6-TBA

TABLE II

SEPARATION OF SOME ACIDIC HERBICIDES

Herbicide	$R_F \times 100$ *in separatory system*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
MCPA	54	57	53	27	60	46	29	48
Mecoprop	68	70	64	38	77	61	46	65
MCPB	80	62	70	40	79	66	36	61
2,4-D	37	45	46	17	43	39	16	43
Dichlorprop	61	64	60	28	65	56	35	58
2,4-DB	76	52	67	32	69	61	27	53
2,4,5-T	39	57	48	21	43	41	24	53
Fenoprop	63	70	60	33	64	59	48	68
Benazolin	36	42	49	21	27	35	14	40
Dicamba	44	70	53	33	52	48	45	67
2,3,6-TBA	35	70	44	30	50	42	51	66
Picloram	—	—	21	2	—	—	—	—

Separatory systems no.	*Adsorbent*	*Mobile solvent*
1	Silica gel–kieselguhr, 2:3	Chloroform–hexane–acetic acid, 66:33:1
2	Silica gel–kieselguhr, 2:3	Hexane–ethyl acetate–formic acid, 80:20:0.4
3	Silica gel	Chloroform–acetic acid, 19:1
4	Silica gel	Hexane–acetone–acetic acid, 18:1:1
5	Silica gel–kieselguhr, 1:1	Hexane acetone–acetic acid, 18:1:1
6	Silica gel	Hexane–chloroform–acetic acid, 13:6:1
7	Silica gel–kieselguhr, 2:3	Hexane–ethyl acetate–formic acid, 150:15:0.2
8	Silica gel	Hexane–ethyl acetate–formic acid, 60:30:0.1

Acidic herbicides

The acidic herbicides have been studied in greatest detail in view of the many available combinations of the active ingredients (Table I). A large number of separatory systems were studied, using silica gel, alumina and kieselguhr, separately and in admixture, as stationary phases (250-μm thick) with solvent mixtures containing acetic or formic acid as mobile phases. The spots were visualized either by spraying with ethanolic silver nitrate solution and irradiating with UV light[2] or by observing the plate under UV light after applying a spray of β-methylumbelliferone.

The systems which appeared to be most promising in regard to their ability to separate these herbicides are listed in Table II. Layers containing alumina were generally unsatisfactory, as tailing or streaking occurred. It was found that by suitable choice of system, any of the common herbicide mixtures[9] could be separated into its components. The use of a mobile phase[13] consisting of ethyl acetate, hexane and formic acid (20:80:0.4) with chromatoplates comprising Silica Gel G and Kieselguhr G (2:3) proved particularly useful in that a partial reversal of the usual R_F order was observed. Combination of this system with a second development at right angles with chloroform, hexane and acetic acid (66:33:1) gave a two-dimensional chromatographic system which separated completely nine out of the eleven compounds studied (Fig. 1). Benazolin and 2,4-D were only partially resolved but these do not, at present, occur together in formulations. Thus, by employing either this two-dimensional technique or the two systems separately, in conjunction with suitable known standard materials, the identity of the components of any of the usual mixed

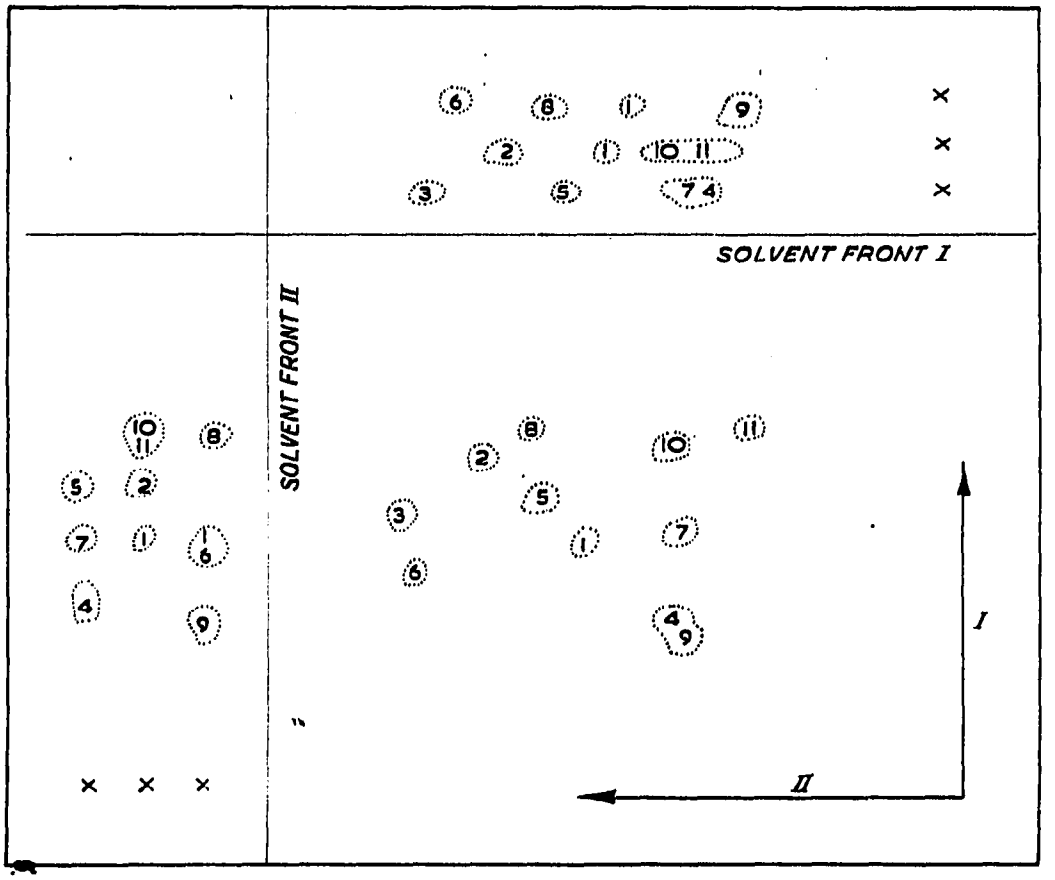

Fig. 1. Two-dimensional chromatogram of acidic herbicides. Adsorbent: silica gel–kieselguhr (2:3). Mobile solvents: (I) hexane–ethyl acetate–formic acid (80:20:0.4); (II) chloroform–hexane–acetic acid (66:33:1). Herbicides: 1 = MCPA, 2 = mecoprop, 3 = MCPB, 4 = 2,4-D, 5 = dichlorprop, 6 = 2,4-DB, 7 = 2,4,5-T, 8 = fenoprop, 9 = benazolin, 10 = dicamba, 11 = 2,3,6-TBA.

formulations can be verified. Suitable specimens for chromatographic study can be obtained from technical materials or formulated products by carrying out the usual ether extraction procedures, after hydrolysis of ester formulations with lithium hydroxide, as described in the methods for determining 'Total Organic Acid' content[10].

In using thin-layer chromatography for the identification of any compound by comparison of its R_F value with that given by a standard material, it is always advisable to apply the sample and standard to the same chromatoplate to obtain simultaneous development under identical conditions. Otherwise, there may be some variation in R_F value from plate to plate, owing to changes in laboratory humidity, in layer activation or to slight differences in mobile solvent composition. This was found to be especially true in the case of these particular herbicides when acidic solvent mixtures were used as mobile phases. Appreciable day to day variation of R_F value was observed with these compounds, although the relative degrees of separation remained similar; the figures quoted in Table II are therefore to be taken only as examples of observed R_F values.

The semi-quantitative use of thin-layer chromatography[5] is best carried out using a chromatographic system which gives an R_F value in the range of 0.3 to 0.6

for the compound under study. An estimate of the quantity of each component of a mixture may be made by comparing the size of the spots obtained from the sample with the size of spots given by known quantities of the identified herbicides. A range of standards of 2, 4, 6, 8, 10 and 12 μg, each applied in 2 μl of solvent to the same chromatoplate as the sample, is suitable for this purpose, taking 2 μl of such a solution of the sample extract as would be expected to contain about 10 μg of total organic acids. Application of the colorimetric procedure of ERNE[13] did not prove satisfactory for quantitative purposes owing to variable interference due to the layer adsorbents used.

Nitrogenous herbicides

Several two- or three-component mixtures containing carbamates, substituted ureas or triazine herbicides are available as commercial formulations[9] (Table I). Fortunately, the thin-layer chromatographic systems required for these compounds are similar in nature though the mobile phases are intrinsically different from those considered for the acidic herbicides. Of the separatory systems studied for these compounds, Table III lists those that were found to be most suitable for the mixtures encountered[9]; consistent R_F values were observed with these nitrogenous compounds in contrast to the varied values given by the acidic herbicides. Silica gel chromatoplates were generally applicable and dichloromethane proved to be the most useful

TABLE III

SEPARATION OF SOME NITROGENOUS HERBICIDES

Stationary phase: Silica Gel G.

Compound	$R_F \times 100$ *in mobile phase*			
	1[b]	2[c]	3[d]	4[e]
Chlorbufam	78	47	64	90
Chlorpropham	79	60	72	86
Propham	68	60	70	87
Cycluron	2	10	29	19
Diuron	12	12	32	50
Fenuron	5	8	25	31
Linuron	50	31	51	73
Monolinuron	37	31	46	67
Monuron	8	9	27	40
Atrazine	3	36	—	34
Methoprotryne	0	31	—	22
Prometryne	6	55	—	57
Simazine	2	34	—	41
Bromacil	5	29	—	50
Bromoxynil	21[a]	5	—	41[a]
Ioxynil	34[a]	8	—	45[a]
Dimexan	90	75	80	92
Dinoseb	61[a]	11[a]	—	66[a]
Endothal	0	0	0	0

[a] Appreciable tailing of spot.
[b] Dichloromethane.
[c] Hexane–acetone, 5:1.
[d] Hexane–acetone, 7:3.
[e] Chloroform–nitromethane, 1:1.

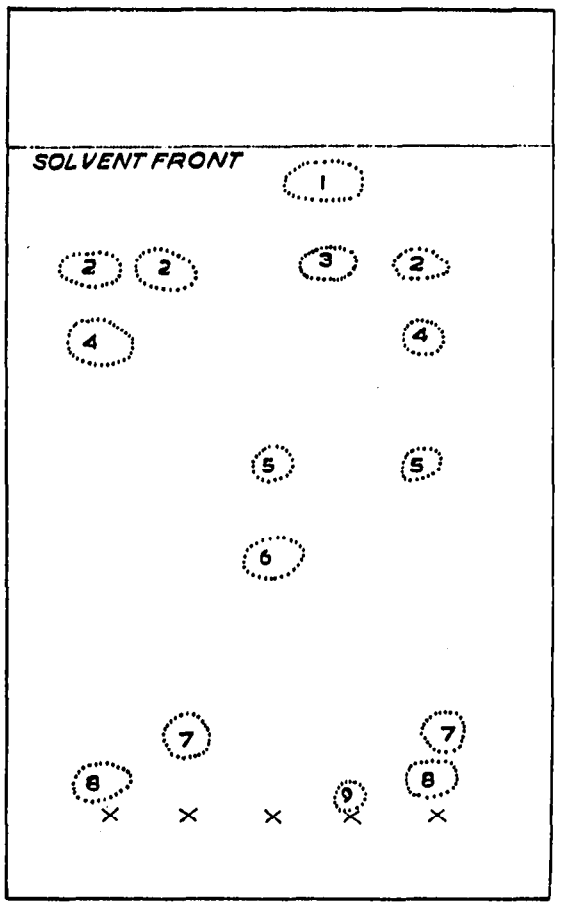

Fig. 2. Separation of some carbamate and substituted urea herbicides. Adsorbent: Silica Gel G. Solvent: dichloromethane. Herbicides: 1 = dimexan, 2 = chlorpropham, 3 = chlorbufam, 4 = propham, 5 = linuron, 6 = monolinuron, 7 = diuron, 8 = fenuron, 9 = cycluron.

mobile phase (Fig. 2). The chromatograms were visualized by spraying with a solution of dichlorofluorescein in acetone and exposing the layer to bromine vapour, upon which pink spots were observed on a white background. Alternatively, the use of a ninhydrin spray, after hydrolysis with hydriodic acid[14] combines the benefits of selective colour formation with chromatogram visualization. Thus the carbamates, giving mauve spots, are distinguished from the ureas, which give pink or reddish-brown spots, while the triazines do not respond to this treatment. Calcofluor R White has also been used[5] to distinguish the carbamates, which yield fluorescent spots, from the substituted ureas, which quench the fluorescence when examined under UV light. Silica Gel GF254, which contains a 'built-in' fluorescent reagent, is also suitable for these separations and its use avoids the need to spray the chromatograms.

The observed R_F values for dimexan, endothal and dinoseb are also included in Table III since these compounds appear in mixtures with some of the compounds under consideration. Similarly, bromacil, bromoxynil and ioxynil are included because they occur in mixtures with certain phenoxyalkanoic acids. All of the acidic herbicides studied remained on the baseline in all systems listed in Table III. Diquat and paraquat are also used together in some formulations but thin-layer chromatography is less suitable for ionic compounds of this nature. Since a satisfactory spectrophoto-

metric method for their determination and identification has been described[15], these compounds were not studied further.

For semi-quantitative purposes, a spot-area comparison procedure can be used, as previously described for the acidic compounds. A graphical method based on areas determined by a 'counting-squares' technique has been described[5], but it is necessarily longer to perform. Suitable extracts from wettable powders may be obtained by solvent treatment. Liquid formulations may be examined by direct application to the chromatoplate of a suitable solution (about 10 μg active ingredient in 2 μl) or after extraction and clean-up of the active ingredients from an aqueous dilution of the formulation.

The thin-layer chromatographic systems described in the paper are equally suitable for the identification of residues of these herbicides, mixtures of which are likely to occur in sample extracts if mixed formulations have been employed. Techniques for the extraction, clean-up and determination of the presence of most of these herbicides in samples of water, soil, etc. have been described[2,4,5].

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