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THIN-LAYER CHROMATOGRAPHY OF ORGANIC SULPHUR COMPOUNDS BY THE MIXED FLUORESCENT MATERIAL METHOD

II. SEPARATION AND QUANTITATION OF DISULPHIDES, THIURAMS, XANTHATES, THIOUREAS AND OTHER ORGANIC SULPHUR COMPOUNDS*

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SUMMARY

The separation and quantitation of organic sulphur compounds were investigated by thin-layer chromatography (TLC) on silica gel layers containing a mixed fluorescent material. The separation of dialkyl disulphides, alkylxanthates and thioureas was performed successfully by reversed-phase TLC using liquid paraffin as the stationary phase. Thiurams and aromatic disulphides were well separated by adsorption TLC. The non-destructive *in situ* quantitation of these compounds on chromatographic plates was achieved by plotting the logarithm of the amount of compound against the square root of the area of the spot revealed under an ultraviolet lamp.

INTRODUCTION

The analysis of organic sulphur compounds by thin-layer chromatography (TLC) or paper chromatography has been performed colorimetrically and fluorimetrically, mainly using various spray reagents. However, in the preceding paper[1] we described a new means of qualitative analysis that permitted the *in situ* non-destructive detection of organic sulphur compounds on TLC plates by using the mixed fluorescent material method[2]. Organic sulphur compounds that absorb UV radiation at 250–400 nm were detected as different coloured spots with limits of detection of 10^{-8}–10^{-11} mole according to their UV absorption spectra and molar absorption coefficients.

So far as the TLC separation of organic sulphur compounds is concerned, there have been reports of studies on asymmetrical heterocyclic disulphides[3], thiolactones[4] and thioureas[5], but the analysis of dialkyl disulphides, thiurams and xanthates has not been reported.

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This paper describes the rapid separation and quantitation of disulphides, thiurams, alkylxanthates and thioureas on silica gel layers containing a mixed fluorescent material. The R_F values of thiolactones and thioesters have also determined.

EXPERIMENTAL

Materials and reagents

The organic sulphur compounds, reagents and solvents used were the same as those specified in the preceding paper[1].

Thin-layer chromatographic plates

All of the experiments were performed with commercially available chromatographic plates (Wakogel FM plate, 10 × 5 cm: Wako, Osaka, Japan) which contained 89% of silica gel, 1% of starch and 7.9% of a mixture of three inorganic fluorescent additives[1].

When reversed-phase TLC was performed, the plates were dipped in a 5% solution of liquid paraffin in light petroleum (b.p. 30–70) for 10 sec, then air dried for 15 min at room temperature (25).

Chromatographic procedure for separation studies

Stock solutions of various organic sulphur compounds of concentration 50 m*M* were prepared with appropriate solvents and a 1 µl aliquot, corresponding to $5 \cdot 10^{-8}$ mole of the compound, was applied with a micro-pipette ("Microcaps", Drummond, Broomall, Pa., U.S.A.) on to 0.8 cm of the lower edge of a TLC plate. The subsequent procedures for the separation and detection of the compounds were performed as described in the preceding paper[1].

Solvent systems

(1) Stationary phase, 5% liquid paraffin; mobile phase, methanol.
(2) Dioxane–methanol (1:3).
(3) Benzene.
(4) *n*-Butanol–acetic acid–water (5:2:3).
(5) *n*-Propanol–28% ammonia (7:3).
(6) Ethyl acetate–*n*-hexane (1:6).
(7) Benzene–dioxane–28% ammonia–water saturated with sodium tetraborate (2:30:0.5:2).
(8) Stationary phase, 5% liquid paraffin; mobile phase, 5% ammonia solution.
(9) Stationary phase, 5% liquid paraffin; mobile phase, 10% sodium acetate solution.
(10) Stationary phase, 5% liquid paraffin; mobile phase, chloroform.
(11) Stationary phase, 5% liquid paraffin; mobile phase, water.

Procedure for quantitation of compounds

Stock solutions of representative members of several classes of organic sulphur compounds were used as follows:

(a) Dialkyl disulphides: 100 m*M* di-*tert*.-hexyl disulphide in ethanol.

(b) Aromatic disulphides: 10 m*M* 2,2′-dibenzothiazolyl disulphide in chloroform–carbon disulphide (1:1).

(c) Thiurams: 100 m*M* tetramethylthiuram monosulphide in chloroform and 100 m*M* tetraethylthiuram disulphide in chloroform.

(d) Xanthates: a mixture of 100 m*M* potassium ethyl xanthate and 100 m*M* potassium *n*-butyl xanthate in 5% ammonia solution.

(e) Thioureas: a mixture of 20 m*M* 1,1-diphenyl-2-thiourea, 20 m*M* 1,3-diphenyl-2-thiourea and 20 m*M* N,N′-dicyclohexylthiourea in chloroform.

Standard solutions were prepared from the corresponding stock solutions using the same solvents, and 1 μl of each standard solution was applied as a circular spot on to 0.8 cm of the lower edge of a chromatographic plate by means of the micropipette. Usually six or seven spots were applied on a plate. After development for a distance of 8.5–9 cm with one of the above solvent systems, each plate was air dried and the spots of the compound(s) present were made visible with a Pan UV lamp[1]. The shapes of the spots were traced from the back of the plate on the surface of the glass and were then copied on to a sheet of thick paper. The area of each spot was calculated by comparison of its weight with that of 100 mm^2 of the paper. The logarithm of the amount (nanomoles) of sample applied was plotted against the square root of the area (square millimetres) of spot formed[6].

RESULTS

Separation of disulphides

The separation of dialkyl disulphides by adsorption TLC was generally un-

TABLE I

R_F VALUES OF DIALKYL DISULPHIDES

Compound	*$R_F \times 100$*		*R_M****
	*I**	*II***	
(A) tert.-Homologues			
$[(CH_3)_3C(CH_2)_{10}\text{-}S]_2$	65	4	+ 1.38
$[(CH_3)_3C(CH_2)_8\text{-}S]_2$	67	9	+ 1.01
$[(CH_3)_3C(CH_2)_4\text{-}S]_2$	74	22	+ 0.55
$[(CH_3)_3C(CH_2)_2\text{-}S]_2$	75	28	+ 0.41
$[(CH_3)_3C\text{-}CH_2\text{-}S]_2$	75	37	+ 0.23
$[(CH_3)_3C\text{-}S]_2$		44	+ 0.11
(B) n-Homologues			
$[CH_3(CH_2)_6\text{-}S]_2$	75	24	+ 0.50
$[CH_3(CH_2)_4\text{-}S]_2$		42	+ 0.14
$[CH_3(CH_2)_3\text{-}S]_2$		50	± 0.00
$[CH_3(CH_2)_2\text{-}S]_2$		54	− 0.07
(C) Others			
$[(CH_3)_2CH(CH_2)_2\text{-}S]_2$		47	
$[(CH_3)_2CH\text{-}CH_2\text{-}S]_2$		49	
$[CH_2{=}CH\text{-}CH_2\text{-}S]_2$		65	

* I: Solvent system 2.

** II: Solvent system 1.

*** Obtained with solvent system 1.

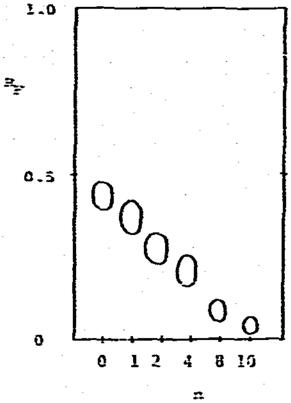


Fig. 1. Separation of symmetrical di-*tert.*-dialkyl disulphides, $[(CH_3)_3C(CH_2)_nS\text{-}]_2$. Stationary phase, 5% liquid paraffin on Wakogel FM plate; mobile phase, methanol; developing time, 60 min (25°).

successful with many solvents, dioxane–methanol (1:3) giving the best resolution (Table I). However, satisfactory results were obtained by reversed-phase partition chromatography using 5% liquid paraffin as the stationary phase and methanol as the mobile phase. As a typical example, the separation of di-*tert.*-dialkyl disulphides is shown in Fig. 1, and a linear relationship was observed between the R_M values [where $R_M = \log(1/R_F - 1)$] and the number of carbon atoms (Martin relationship[7]), as shown in Fig. 2.

Fifteen aromatic disulphides were successfully separated on silica gel layers with benzene as the developing solvent (Table II), and Fig. 3 shows typical chromatograms.

The R_F values of thirteen polar aliphatic disulphides, two S-sulphonic acids and other related compounds in *n*-butanol–acetic acid–water (5:2:3) and *n*-propanol–

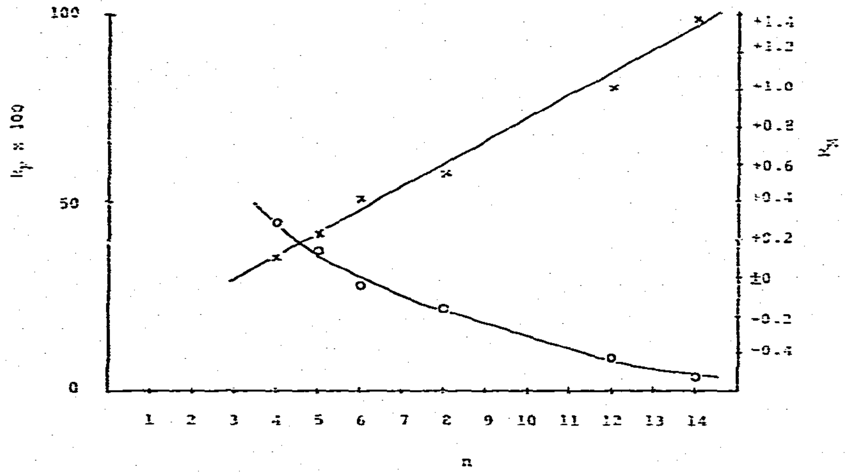


Fig. 2. R_F (○) and R_M (×) values of symmetrical di-*tert.*-dialkyl disulphides, $C_nH_{2n+1}SSC_nH_{2n+1}$. TLC conditions as in Fig. 1.

TABLE II

R_F VALUES OF AROMATIC POLYSULPHIDES

Developing agent, benzene; TLC plate, Wakogel FM; developing time, 25 min (25°).

No.	*Compound*	$R_F \times 100$
1	Uridine-4-disulphide	0
2	Thiamine disulphide	0
3	5,5′-Dithiobis(2-nitrobenzoic acid)	0
4	2,2′-Dihydroxy-6,6′-dinaphthyl disulphide	2
5	4,4′-Dithiodianiline	6
6	2,2″-Dithiodibenzanilide	12
7	Diquinolyl-8,8′-disulphide	28
8	2,2′-Dithiodianiline	28
9	Bis(2,4-dinitrophenyl) disulphide	42
10	2,2′-Dibenzothiazolyl disulphide	52
11	*p,p*′-Dinitrophenyl disulphide	74
12	*o,o*′-Dinitrophenyl disulphide	78
13	Benzyl disulphide	84
14	2-Naphthyl disulphide	86
15	Diphenyl disulphide	87
16	Bis(2,4,5-trichlorophenyl) disulphide	87
17	Bis(2,4,5-trichlorophenyl) trisulphide	88

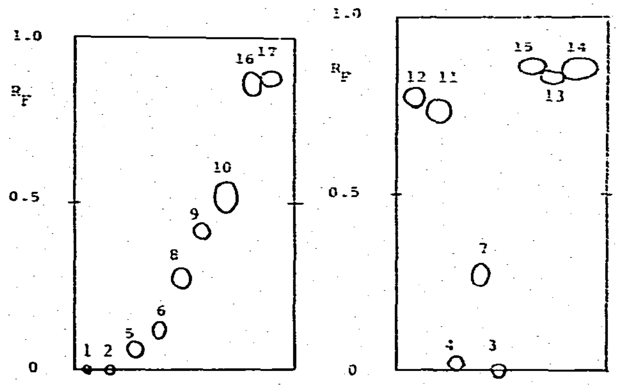


Fig. 3. Separation of aromatic polysulphides. TLC conditions and numbers as in Table II.

28% ammonia (7:3) are given in Table III. Table III and Fig. 4 show that these two solvent systems are suitable for the separation of polar aliphatic disulphides and S-sulphonic acids. The possibility of the degradation of the disulphide bond by the strongly alkaline ammoniacal solvent was examined using four disulphides containing primary amino groups, *viz.* cystamine, formamidine disulphide, L-cystine and homocystine. Amounts of $5 \cdot 10^{-8}$ mole of each compound were separately developed with *n*-propanol–28% ammonia (7:3) and detected first by the mixed fluorescent material method[1] and then with ninhydrin[8]. The spots developed with ninhydrin corresponded completely to those revealed by UV light and their R_F values were different from those of the corresponding thiols. These results indicate that degradation of the disulphides into the corresponding thiols or cleavage of the disulphide bond is not likely, although

TABLE III

R_F VALUES OF POLAR ALIPHATIC DISULPHIDES AND OTHER COMPOUNDS

No.	*Compound*	$R_F \times 100$	
		*I**	*II***
	(a) Acidic disulphides		
1	Dithiodiacetic acid	64	34
2	α,α′-Dithiodipropionic acid	84	44
3	β,β′-Dithiodipropionic acid	78	44
4	Lipoic acid	91	68
	(b) Basic disulphides		
5	Formamidine disulphide·2HCl	78	73
6	Cystamine	36	81
7	β-Alethine	49	70
	(c) Amino acids		
8	L-Cystine	35	29
9	Homocystine	47	34
10	L-Glutathione oxidized	21	8
	(d) Pantethine analogues		
11	Pantethine	78	86
12	D-Pantethine-4′,4″-diphosphate	40	2
13	Di-D-pantothenoyl-L-cystine	60	46
14	Pantetheine-S-sulphonic acid	65	70
15	4′-Phosphopantetheine-S-sulphonic acid	36	0
	(e) Others		
16	Morpholine N,N′-disulphide	94	89
17	Xanthane hydride	89	71
18	Sulphur	94***	94***

* I: Solvent system 4.
** II: Solvent system 5.
*** Tailing.

oxidative modification of the disulphide bond in alkaline medium to give thiosulphinates (RSOSR), thiosulphonates (RSO_2SR), etc., may be possible.

Separation of thiurams

The complete separation of several thiurams was achieved by adsorption TLC using benzene (Fig. 5) or ethyl acetate–*n*-hexane (1:6) as the developing solvent, and R_F values are given in Table IV.

Separation of xanthates

The R_F values of seven alkylxanthates of C_1–C_5 alcohols obtained in three solvent systems and also the R_M values are shown in Table V. As in the separation of dialkyl disulphides, the separation of alkylxanthates by adsorption TLC was incomplete, whereas they were completely separated by reversed-phase TLC using 5% liquid paraffin as the stationary phase and 5% ammonia or 10% sodium acetate solution as the mobile phase. As already pointed out by Gasparič and Borecký[9], marked degradation of xanthates occurred in neutral solvent systems, and even in 10% sodium acetate solution trace amounts of degradation products, which were distinguishable

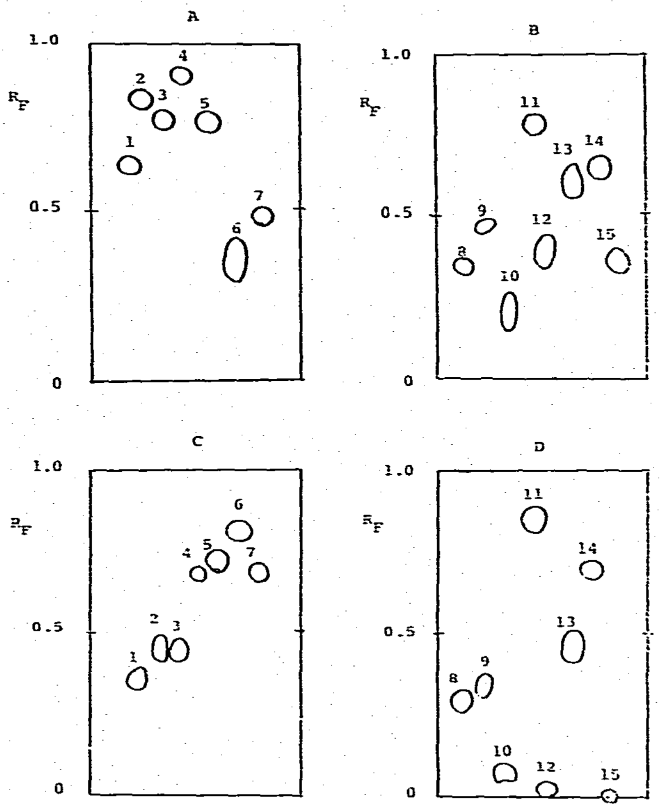


Fig. 4. Separation of polar aliphatic disulphides and related compounds. Plates A and B were developed with solvent system I and Plates C and D with the solvent system II. TLC conditions and numbers for solvent systems and compounds as in Table III.

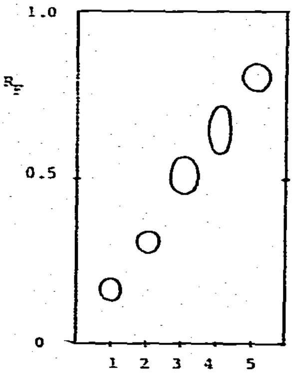


Fig. 5. Separation of thiuram compounds. 1, Tetramethylthiuram monosulphide; 2, tetramethylthiuram disulphide; 3, tetraethylthiuram disulphide; 4, tetra-*n*-buthylthiuram disulphide; 5, dipentamethylenethiuram tetrasulphide. Developing agent, benzene; TLC plate, Wakogel FM; developing time, 25 min (25°).

TABLE IV

R_F VALUES OF THIURAM COMPOUNDS

Compound	*$R_F \times 100$*	
	*I**	*II***
Tetramethylthiuram monosulphide	17	16
Tetramethylthiuram disulphide	31	26
Tetraethylthiuram disulphide	52	47
Tetra-*n*-butylthiuram disulphide	81	84
Dipentamethylenethiuram tetrasulphide	65	59

* I: Solvent system 3.
** II: Solvent system 6.

TABLE V

R_F VALUES OF ALKYLXANTHATES

Compound	*$R_F \times 100$*			*R_M*§
	*I**	*II***	*III****	
CH_3OCSSK	29	74	91	-1.01
C_2H_5OCSSK	35	74	81	-0.63
n-C_3H_7OCSSK	45	55	62	-0.21
iso-C_3H_7OCSSK	44	61	65	-0.27
n-C_4H_9OCSSK	52	34	39	$+0.19$
iso-C_4H_9OCSSK	49	37	41	$+0.16$
n-$C_5H_{11}OCSSK$	52	15	22	$+0.55$

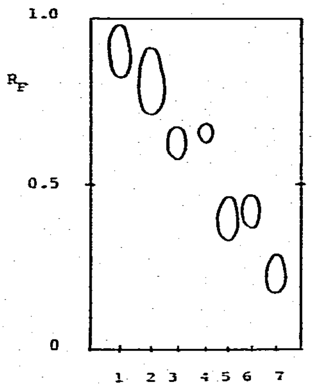
* I: Solvent system 7.
** II: Solvent system 9.
*** III: Solvent system 8.
§ Obtained with solvent system 8.

from the bluish green colour of xanthates by their red colour under the Pan UV lamp, were also observed. However, no degradation of alkylxanthates was observed in 5% ammonia solution (Fig. 6). The relationship between the R_F and R_M values obtained by reversed-phase TLC and the number of carbon atoms in *n*-alkylxanthates is shown in Fig. 7, which indicates the adaptation of the separation system used to the Martin relationship[7].

Separation of thioureas

The separation of thioureas was unsuccessful on silica gel layers but satisfactory results were obtained on 5% liquid paraffin-coated silica gel layers. Eleven thioureas were separated on such layers by reversed-phase TLC using water as the mobile phase, but a better separation was achieved with chloroform as the mobile phase (Table VI, Fig. 8).

On liquid paraffin-coated plates, a relationship was observed between the R_F or R_M values and the number of carbon atoms in di-*n*-alkylthioureas (Fig. 9).



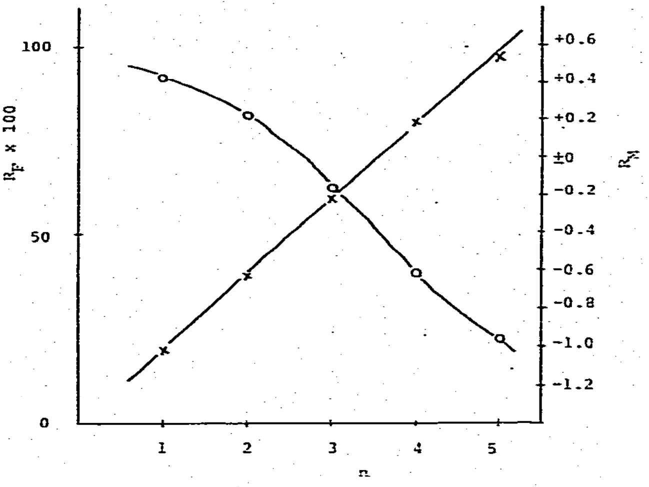


Fig. 6. Separation of alkylxanthates. 1, CH_3OCSSK; 2, C_2H_5OCSSK; 3, *n*-C_3H_7OCSSK; 4, *iso*-C_3H_7OCSSK; 5, *n*-C_4H_9OCSSK; 6, *iso*-C_4H_9OCSSK; 7, *n*-$C_5H_{11}OCSSK$. Stationary phase, 5% liquid paraffin on Wakogel FM plate; mobile phase, 5% ammonia solution; developing time, 35 min (25°).

Fig. 7. R_F (○) and R_M (×) values of *n*-alkylxanthates, $C_nH_{2n+1}OCSSK$. TLC conditions as in Fig. 6.

TABLE VI

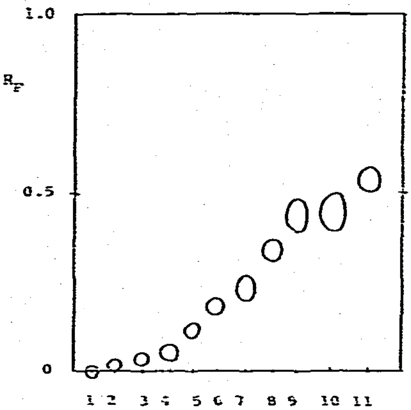
R_F VALUES OF THIOUREA DERIVATIVES

No.	*Compound*	*R_F × 100*		*R_M*	
		*I**	*II***	*I**	*II***
1	Thiourea	0	95	∞	−1.28
2	Thiosemicarbazide·2HCl	1	86		
3	4-Methylthiosemicarbazide	3	67		
4	1,3-Dimethylthiourea	6	60	+1.20	−0.18
5	1-Acetyl-2-thiourea	12	67		
6	1,3-Diethylthiourea	20	32	+0.60	+0.33
7	1,1-Diphenyl-2-thiourea	23	0		
8	N,N′-Diisopropylthiourea	34	8		
9	1,3-Diphenyl-2-thiourea	44	0		
10	N,N′-Di-*n*-butylthiourea	44	0	+0.11	∞
11	N,N′-Dicyclohexylthiourea	55	0		

* I: Solvent system 10.
** II: Solvent system 11.

Separation of thioesters and thiolactones

The R_F values of several organic sulphur compounds containing the –S–CO– group, *i.e.* thioesters and thiolactones, are given in Table VII.



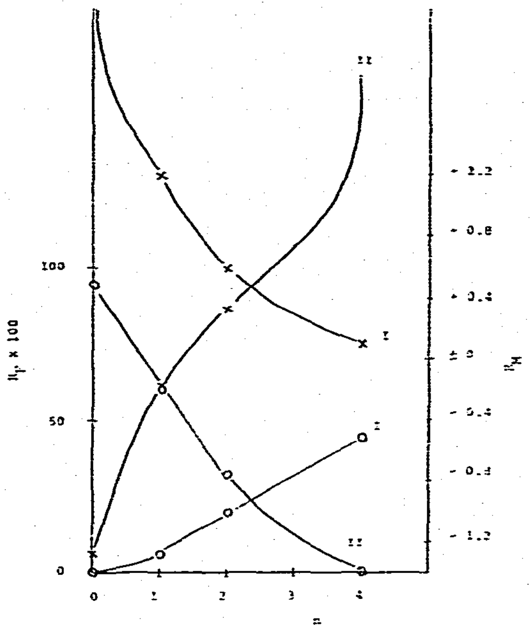


Fig. 8. Separation of thiourea derivatives. The numbers indicate the thiourea derivatives listed in Table VI. Stationary phase, 5% liquid paraffin on Wakogel FM plate; mobile phase, chloroform.

Fig. 9. R_F (○) and R_M (×) values of symmetrical *n*-alkylthioureas, $(C_nH_{2n+1}NH)_2C=S$. Stationary phase, 5% liquid paraffin on Wakogel FM plate; mobile phase, chloroform (I) or water (II).

TABLE VII

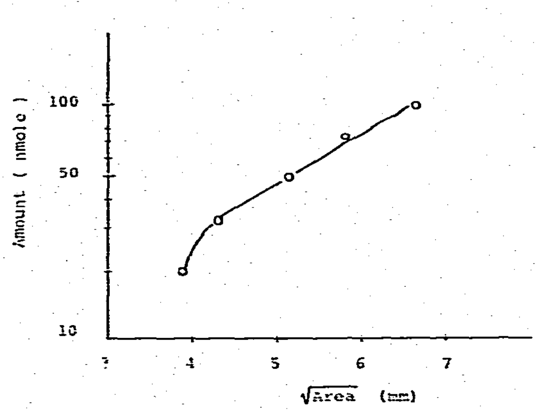
R_F VALUES OF THIOESTERS AND THIOLACTONES

Developing agent, *n*-butanol-acetic acid-water (5:2:3); TLC plate, Wakogel FM.

Compound	*$R_F \times 100$*
S-Acetylglutathione	41
Acetylthiocholine iodide	22
S-Carbamyl-L-cysteine	42
1-Thioglucopyranose pentaacetate	87
DL-Homocysteine thiolactone·HCl	61
N-Acetyl-DL-homocysteine thiolactone	73

Quantitation of disulphides, thiurams, xanthates and thioureas

Non-destructive quantitative analyses of some classes of organic sulphur compounds on thin layers containing the mixed fluorescent material were successfully performed using the best separation conditions for the respective classes described above. A linear relationship was obtained between the logarithm of the amount of compound and the square root of the area of the spot formed in both partition and adsorption TLC. The limits of detection of di-*tert*.-hexyl disulphide (Fig. 10), 2,2'-dibenzothiazolyl disulphide (Fig. 11), thiurams (tetramethylthiuram monosulphide



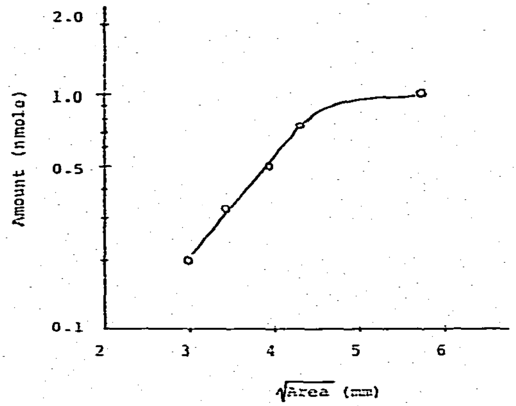


Fig. 10. Working curve for di-*tert*.-hexyl disulphide. TLC conditions as in Fig. 1.

Fig. 11. Working curve for 2,2'-dibenzothiazolyl disulphide. TLC conditions as in Table II.

and tetraethylthiuram disulphide: Fig. 12), *n*-alkylxanthates (potassium ethylxanthate and potassium *n*-butylxanthate: Fig. 13) and thioureas (1,1-diphenyl-2-thiourea, 1,3-diphenyl-2-thiourea, N,N'-diisopropylthiourea and N,N'-dicyclohexylthiourea: Fig. 14) were 3.3–10·10^{-8}, 2–7.5·10^{-10}, 4–20·10^{-9}, 1.65–5·10^{-9} and 2–15·10^{-9} mole, respectively.

DISCUSSION

The Martin relationship[7], in which R_M values plotted against carbon number should give a straight line, has been shown to be obeyed in a large number of instances[10]. With organic sulphur compounds, Prinzler *et al.*[11] have demonstrated that this rule holds true for dialkyl sulphides and alkyl aryl sulphides. A similar relationship was also observed in this investigation in the reversed-phase partition chromatography of symmetrical di-*tert*.-alkyl disulphides (Fig. 1) and *n*-alkyl xanthates (Fig. 6).

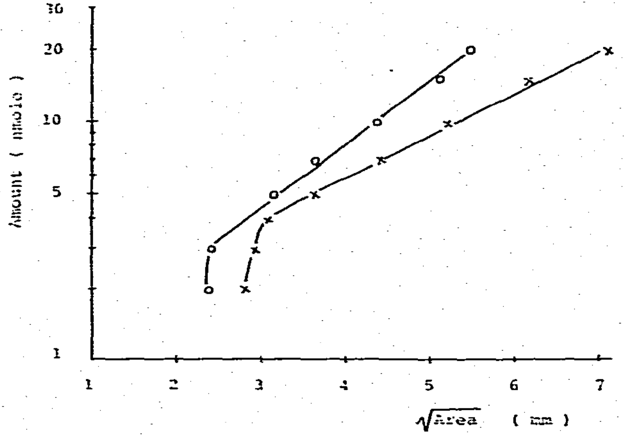


Fig. 12. Working curves for tetramethylthiuram monosulphide (○) and tetraethylthiuram disulphide (×). TLC conditions as in Fig. 5.

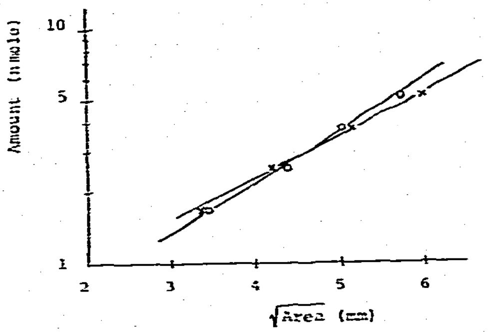


Fig. 13. Working curves for potassium ethylxanthate ( ) and potassium *n*-butylxanthate (×). TLC conditions as in Fig. 6.

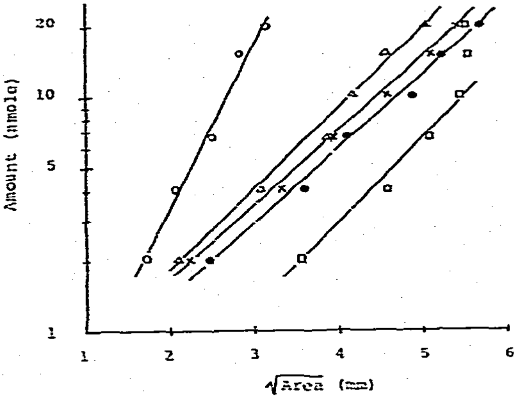


Fig. 14. Working curves for thioureas. , 1,3-Dimethylthiourea; ●, 1,1-diphenyl-2-thiourea; , 1,3-diphenyl-2-thiourea; △, N,N′-diisopropylthiourea; ×, N,N′-dicyclohexylthiourea. TLC conditions as in Fig. 8.

In this paper, the utility of the mixed fluorescent material method in quantitative analysis has been demonstrated for the first time. The sensitivity of this method bears comparison only with that of one TLC method reported for maneb and zineb (0.6 μg)[12] and also with that of an assay method for disulphides in solution using 5,5′-dithiobis(2-nitrobenzoic acid) ($9 \cdot 10^{-9}$ mole)[13].

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