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

I. DETECTION OF VARIOUS CLASSES OF COMPOUNDS*

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SUMMARY

Organic sulphur compounds were detected on silica gel layers containing a mixed fluorescent material. Disulphides, thioureas, isothioureas, thioamides, thiolactones, thioesters, thiurams, dithiocarbamates, xanthates, S-sulphonic acids, thiocyanates, isothiocyanates and organothiophosphorus compounds were detected as coloured spots in amounts of 10^{-8}–10^{-11} mole according to their ultraviolet absorption spectra and molar absorption coefficients. Thiols, sulphides, sulphonic acids, sulphinic acids, sulphates, sulphamates, sulphones and sulphoxides did not give any coloured spots in amounts of 10^{-7} mole. When the sulphur was replaced with oxygen, the positive compounds above were no longer detected even in amounts of 10^{-7} mole.

INTRODUCTION

Organic sulphur compounds have been detected on chromatograms mainly by using various spray reagents of relatively low specificity. The spraying method often requires troublesome procedures and lacks reproducibility, whereas the qualitative analysis of ultraviolet (UV)-absorbing compounds by the mixed fluorescent material method, recently developed by Tamura[1], requires no complicated procedures and is non-destructive unless the compounds are sensitive to UV light. Some classes of organic sulphur compounds, such as disulphides, thiurams, thioureas and xanthates, may absorb UV light at wavelengths above 250 nm, and the mixed fluorescent material method may therefore provide a unique analytical method for these compounds, especially disulphides, whose detection on chromatograms in an intact form, *i.e.*, without reduction to thiols or oxidation of the disulphide bonds, has not been reported.

This paper describes the *in situ* non-destructive detection of organic sulphur compounds on silica gel layers containing a mixed fluorescent material.

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EXPERIMENTAL

Reagents and solvents

L-Glutathione (oxidized), S-carbamyl-L-cysteine, S-acetylglutathione, DL-homocysteine hydrochloride, 1-thioglucopyranose pentaacetate and D-glucose-6-sulphate potassium salt were purchased from Seikagaku Kogyo Co., Tokyo, Japan, and diquinolyl-8,8′-disulphide and 2,2′-dihydroxy-6,6′-dinaphthyl disulphide from Wako, Osaka, Japan. 2-Naphthyl disulphide was obtained from Eastman-Kodak, Rochester, N.Y., U.S.A., and D(+)-biotin from E. Merck, Darmstadt, G.F.R. Uridine-4-disulphide and D-pantoyltaurine calcium salt were purchased from Sigma, St. Louis, Mo., U.S.A. Pantethine, D-pantethine 4′,4″-diphosphate and di-D-pantothenoyl-L-cystine were provided by Daiichi Seiyaku Co., Tokyo, Japan. Fenthion (Baytex), ethion, disyston and mesurol were kindly provided by Nihon Tokushu Noyaku Seizo Co., Tokyo, Japan. Pantetheine-S-sulphonic acid and 4′-phosphopantetheine-S-sulphonic acid were prepared in this laboratory. All other reagents and solvents were purchased from Tokyo Kasei Kogyo Co., Tokyo, or Kanto Chemical Co., Tokyo, Japan.

Apparatus

A Pan UV lamp (Type PUV-1A, Tokyo Kogaku Kikai, Tokyo, Japan) was used, which provides continuous UV radiation at 250–400 nm through an available surface area (7.5 × 4.5 cm) of luminescence.

Thin-layer chromatographic plates

Thin-layer chromatography (TLC) was performed with commercially available chromatographic plates (Wakogel FM plate, 10 × 5 cm; Wako). The layer (250 μm thickness) on the plates contained, in addition to silica gel and starch, three inorganic fluorescent additives, $Sr_2P_2O_7/Sn$ (λ_{max}, 260 nm, blue fluorescence), Zn_2SiO_4/Mn (λ_{max}, 280 nm, green fluorescence) and YVO_4/Eu (λ_{max}, 330 nm, red fluorescence), in a ratio of 20:5:1.

Solvent systems

The two solvent systems used were (A) methanol–dioxane (1:1) and (B) *n*-propanol–28% ammonia (7:3). Most of the test compounds were developed with the neutral solvent system A with R_F values of above 0.5. Xanthates were developed with the alkaline solvent system B in order to prevent their decomposition.

Chromatographic procedure

Stock solutions of test compounds of concentration 0.1 *M* were prepared with appropriate solvents (water, methanol, ethanol, ethyl acetate, acetone, carbon disulphide, chloroform, benzene, 0.2 *N* hydrochloric acid, 0.2 *N* sodium hydroxide solution, 5% ammonia solution and their mixtures) and were diluted as required with the same solvents. A volume of 1 μl of a stock solution or its dilution 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. After air-drying, the plate was developed for a distance of 8.5–9 cm in a small chromatographic chamber (ambient temperature *ca.*

25°). The developed plate was dried with a stream of air using a hair-dryer and was viewed from the back under UV light from the Pan UV lamp in the dark[2].

The compounds that gave no coloured spots in amounts of $1 \cdot 10^{-7}$ mole were designated as negative compounds.

RESULTS

The colours and the limits of detection of various classes of organic sulphur compounds on Wakogel FM plates are summarized in Table I, together with $\lambda_{max.}$ and log ε values taken from the literature. In general, disulphides, thioureas, isothioureas, thiocarboxylic acid derivatives (thiol acids, thioamides, thiolactones and thioesters), thiocarbamic acid derivatives (thiuram compounds and dithiocarbamates), xanthates, S-sulphonic acids, organothiophosphorus compounds, thiocyanates and isothiocyanates were detected as coloured spots in amounts of less than $1 \cdot 10^{-7}$ mole. On the other hand, thiols, sulphides, sulphonic acids, sulphinic acids, sulphates, sulphamates, sulphones and sulphoxides did not give any coloured spots at this level.

As expected, the positive compounds in Table I were exclusively those with $\lambda_{max.}$ values above 250 nm and the sensitivities of detection depended mainly upon their log ε values. The colours of the positive compounds on the TLC plates under UV light differed according to their UV spectra. The characteristic colours of the sulphur-containing functional groups were altered by the presence of a linked aromatic ring, while an additive colour was developed by the presence of a separated aromatic ring. A representative example is aromatic disulphides: diphenyl disulphide and 2-naphthyl disulphide were visible as violet spots, benzyl disulphide gave red spots. In general, the linkage effect in aromatic disulphides contributed to the increase in sensitivity by a factor of approximately 10–100. In fact, the limits of detection of dialkyl disulphides and polar aliphatic disulphides were 10^{-8}–10^{-9} mole, whereas those of aromatic disulphides, except benzyl disulphide, were 10^{-10}–10^{-11} mole.

When the sulphur was replaced with oxygen, the above compounds were no longer detected, even in amounts of $1 \cdot 10^{-7}$ mole. In view of the UV spectra and the log ε values given in Table II, these results are reasonable: no large absorption above 250 nm was observed.

DISCUSSION

The qualitative analysis of UV-absorbing substances has previously been performed on thin layers with fluorescent additives, the most popular being silica gel F_{254} and $HF_{254+366}$ (Merck). On these layers, however, all types of UV-absorbing substances are rendered visible as dark, uncoloured spots, while on layers containing the mixed fluorescent material used in the present work, different colours occur according to the wavelength of the UV light absorbed by the substances. Thus various organic sulphur compounds have been detected with a clear distinction of colour. For example, although disulphides, thiocyanates, isothiocyanates, thiolactones, thioureas, isothioureas and S-sulphonic acids generally showed a red colour on Wakogel FM plates, the colours of dithiocarbamates and thiuram compounds were reddish violet and that of alkylxanthates was bluish green. The presence of substituted groups that exert electromeric effects on the –SS– bond of disulphides gave rise to clear differences of

TABLE I

LIMITS OF DETECTION OF VARIOUS CLASSES OF ORGANIC SULPHUR COMPOUNDS ON THIN LAYERS CONTAINING MIXED FLUORESCENT MATERIAL

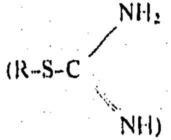
Classification	*Compound*	*Colour**	*Limit of detection*** *(mole)*	$\lambda_{max.}$ *(log ε)* *(nm)*	*Solvent*	*Reference*
(1) Thiols	L-Cysteine	None	$\cdot 10^{-7}$	230 (3.30)	Water (pH 8.32)	3
(RSH)	DL-Homocysteine	None	$\cdot 10^{-7}$			
	Glutathione	None	$\cdot 10^{-7}$	No maxima 220–320	0.03 *N* HCl	4
	Dithiothreitol	None	$\cdot 10^{-7}$			
	n-Decyl mercaptan	None	$\cdot 10^{-7}$			
	n-Dodecyl mercaptan	None	$\cdot 10^{-7}$			
	n-Tetradecyl mercaptan	None	$\cdot 10^{-7}$			
	n-Hexadecyl mercaptan	None	$\cdot 10^{-7}$			
	Thiophenol***	Reddish violet	$1 \cdot 10^{-9}$	236 (3.93), 272 (2.81), 280 (2.84)	Isooctane	4
(2) Polysulphides	*(A) Dialkyl disulphides*					
(RS_nR; $n \geq 2$)	Dimethyl disulphide***	Red	$\cdot 10^{-7}$	203 (3.27), 254.5 (2.53)	Ethanol	4
	Diethyl disulphide***	Red	$\cdot 10^{-7}$	202 (3.32), 251.5 (2.62)	Ethanol	4
	Di-*n*-propyl disulphide***	Red	$1 \cdot 10^{-7}$	250 (2.7)	Ethanol	5
	Di-*n*-butyl disulphide***	Red	$1 \cdot 10^{-8}$	204 (3.32), 251.5 (2.60)	Ethanol	4
	Di-isobutyl disulphide***	Red	$1 \cdot 10^{-8}$			
	Di-*n*-amyl disulphide	Red	$1 \cdot 10^{-8}$	254 (2.7)	Isooctane	3
	Di-*tert.*-butyl disulphide***	Red	$1 \cdot 10^{-8}$			
	Di-*tert.*-amyl disulphide	Reddish violet	$8 \cdot 10^{-9}$			
	Di-isoamyl disulphide	Red	$7 \cdot 10^{-9}$	252 (2.6)	Isooctane	3
	Di-*tert.*-hexyl disulphide	Red	$7 \cdot 10^{-9}$			
	Di-*n*-heptyl disulphide	Reddish pink	$3 \cdot 10^{-9}$			
	Di-*tert.*-octyl disulphide	Reddish pink	$3 \cdot 10^{-9}$			
	Di-*tert.*-dodecyl disulphide	Reddish pink	$3 \cdot 10^{-9}$			
	Di-*tert.*-tetradecyl disulphide	Reddish pink	$3 \cdot 10^{-9}$			
	(B) Polar aliphatic disulphides					
	Dithiodiacetic acid	Red	$7 \cdot 10^{-9}$	None above 250		6
	α,α'-Dithiodipropionic acid	Red	$2 \cdot 10^{-8}$			

β,β′-Dithiodipropionic acid	Red	$2\cdot10^{-8}$	243 (2,73)	Water	7
Lipoic acid	Grey	$3\cdot10^{-9}$	333 (2,18)	Methanol	5
Cystamine	Red	$3\cdot10^{-9}$			
Formamidine disulphide·2HCl	Reddish violet	$5\cdot10^{-9}$			
β-Alethine	Red	$4\cdot10^{-9}$			
L-Cystine	Reddish orange	$3\cdot10^{-9}$	249 (2,53)	0,1 *N* NaOH	4
Homocystine	Reddish orange	$1\cdot10^{-8}$			
D-Pantethine	Red	$8\cdot10^{-9}$			
D-Pantethine 4′,4″-diphosphate	Red	$8\cdot10^{-9}$			
Di-D-pantothenoyl-L-cystine	Red	$8\cdot10^{-9}$			
Morpholine N,N′-disulphide	Reddish violet	$1\cdot10^{-9}$			
(C) Aromatic disulphides					
Diphenyl disulphide	Violet	$5\cdot10^{-10}$	238 (4,2), 270 (3,6), 300 (3,1)		8
Benzyl disulphide	Red	$2\cdot10^{-9}$	221 (4,3)	Isooctane	8
2-Naphthyl disulphide	Violet	$5\cdot10^{-10}$			
Diquinolyl-8,8′-disulphide	Violet brown	$5\cdot10^{-11}$			
2,2′-Dihydroxy-6,6′-dinaphthyl disulphide	Reddish violet	$7,5\cdot10^{-11}$			
o,o′-Dinitrophenyl disulphide	Brown	$1\cdot10^{-10}$	240 (4,37), 351 (3,89)	Ethanol	4
p,p′-Dinitrophenyl disulphide	Bluish green	$5\cdot10^{-11}$	315 (4,33)	Ethanol	4
5,5′-Dithiobis (2-nitrobenzoic acid)	Bluish green	$5\cdot10^{-11}$			
2,2′-Dithiodianiline	Black	$2\cdot10^{-10}$			
4,4′-Dithiodianiline	Black	$1\cdot10^{-10}$			
2,2″-Dithiodibenzanilide	Violet black	$6\cdot10^{-11}$			
Bis(2,4-dinitrophenyl) disulphide	Black	$2,5\cdot10^{-11}$	248 (4,19), 332 (4,03), 410 (3,96)	Ethanol	4
Thiamine disulphide	Reddish violet	$5\cdot10^{-11}$			
Bis(2,4,5-trichlorophenyl) disulphide	Reddish violet	$1\cdot10^{-10}$			
2,2′-Dibenzothiazolyl disulphide	Dark grey	$6,3\cdot10^{-11}$			
Uridine-4-disulphide	Bluish green	$1\cdot10^{-10}$			
(D) Polysulphides and others					
Bis(2,4,5-trichlorophenyl) trisulphide	Reddish violet	$1\cdot10^{-10}$			

(Continued on p. 200)

TABLE I (*continued*)

Classification	*Compound*	*Colour**	*Limit of detection*** *(mole)*	$\lambda_{max.}$ *(log ε)* *(nm)*	*Solvent*	*Reference*
	Sulphur	Dark grey	$2\cdot10^{-10}$			
	Xanthane hydride	Green	$5\cdot10^{-10}$	230 (3.85), 286 (4.28), 310 (3.87), 355 (3.80)	Ethanol	9
	Diallyl disulphide***	Reddish violet	$1\cdot10^{-7}$			
(3) Sulphides (RSR')	*n*-Propyl sulphide	None	$\cdot10^{-7}$			
	n-Butyl sulphide	None	$\cdot10^{-7}$	210 (3.09), 229 (2.16)	Ethanol	4
	n-Amyl sulphide	None	$\cdot10^{-7}$			
	n-Hexyl sulphide	None	$\cdot10^{-7}$			
	n-Heptyl sulphide	None	$\cdot10^{-7}$			
	n-Octyl sulphide	None	$\cdot10^{-7}$			
	n-Nonyl sulphide	None	$\cdot10^{-7}$			
	n-Decyl sulphide	None	$\cdot10^{-7}$			
	L-Methionine	None	$\cdot10^{-7}$	Featureless above 240	Water	4
	DL-Lanthionine	None	$\cdot10^{-7}$			
	Djenkolic acid	None	$\cdot10^{-7}$			
	D(+)-Biotin	None	$\cdot10^{-7}$			
	Mesurol	Red	$8\cdot10^{-10}$			
	Bis(tri-*n*-butyltin) sulphide	Red	$6\cdot10^{-8}$			
	Bacitracin	Blue	$3\cdot10^{-10}$			
	Tetrahydrothiophene	None	$\cdot10^{-7}$	210 (3.04), 239 (1.73)	Ethanol	4
	Thiophene***	Red	$\cdot10^{-7}$	231 (3.85) 323 (4.42)	Ethanol Hexane	4 3
	Thiazole***	Red	10^{-7}			
(4) Thioureas (RR'NCNRR' with S double-bonded to C)	Thiourea	Red	$1\cdot10^{-8}$	235 (4.04)	Ethanol	4
	1,3-Dimethylthiourea	Red	$2\cdot10^{-9}$	238 (4.13), 280	Ethanol	7
	1,3-Diethylthiourea	Red	$1\cdot10^{-9}$			
	1-Acetyl-2-thiourea	Violet red	$1\cdot10^{-9}$	208 (4.04), 275 (4.15)	Ethanol	6
	N,N'-Diisopropylthiourea	Red	$2.5\cdot10^{-9}$			
	N,N'-Di-*n*-butylthiourea	Red	$2.5\cdot10^{-9}$			
	1,1-Diphenyl-2-thiourea	Greyish violet	$4\cdot10^{-10}$			
	1,3-Diphenyl-2-thiourea	Greyish violet	$1\cdot10^{-10}$			

	Thiosemicarbazide·2HCl	Red	$1\cdot10^{-9}$	235 (4,0)	Water	4
	4-Methylthiosemicarbazide	Red	$1\cdot10^{-9}$			
	N,N'-Dicyclohexylthiourea	Red	$1\cdot10^{-9}$			
	2-Thiobarbituric acid	Reddish violet	$8\cdot10^{-10}$	235 (3,74), 285 (4,26)	Ethanol	10
(5) Isothioureas	Methylisothiourea sulphate	Red	$1\cdot10^{-9}$	220 (4,58) 238 (3,74)	pH 7,05 pH 12,0	5 5
 $\left(R-S-C\begin{smallmatrix}NH_2\\NH\end{smallmatrix}\right)$	Ethylisothiourea·HBr	Red	$2\cdot10^{-9}$			
	β-Dimethylaminoethylisothiourea·2HCl	Red	$2\cdot10^{-8}$			
(6) Thiocarboxylic acid derivatives	*(A) Thiol acids*					
	Thioacetic acid	Red	$1\cdot10^{-8}$	245 (2,42)	Water	3
	(B) Thioamides					
	Thioacetamide	Red	$2\cdot10^{-9}$	262 (4,08)	Water	3
	Thiopropionamide	Red	$2,5\cdot10^{-10}$			
	Rubeanic acid	Bluish green	$1\cdot10^{-9}$			
	(C) Thiolactones					
	DL-Homocysteine thiolactone hydrochloride	Red	$3\cdot10^{-9}$			
	N-Acetyl-DL-homocysteine thiolactone	Red	$6\cdot10^{-9}$			
	(D) Thioesters					
	S-Acetylglutathione	Red	$4,8\cdot10^{-9}$	232 (3,76)	Phosphate buffer (pH 6,5)	5
	Acetylthiocholine iodide	Red	$2,5\cdot10^{-10}$			
	1-Thioglucopyranose pentaacetate	Reddish violet	$4\cdot10^{-8}$			
(7) Thiocarbamic acid derivatives	*(A) Thiocarbamates*					
	S-Carbamyl-L-cysteine	Red	$5\cdot10^{-8}$			
	(B) Dithiocarbamates					
	Sodium N,N-dimethyldithiocarbamate	Reddish violet	$1\cdot10^{-8}$			

(Continued on p. 202)

TABLE I *(continued)*

Classification	*Compound*	*Colour**	*Limit of detection** (mole)*	*λ*	*Solvent*	*Reference*
	Sodium N,N-diethyldithiocarbamate	Reddish violet	$2\cdot 10^{-9}$	257 (4.08), 290 (4.11)	Ethanol	4
	(C) Thiurams					
	Tetramethylthiuram disulphide	Reddish violet	$4\cdot 10^{-10}$	270 (4.0)		4
	Tetraethylthiuram disulphide	Reddish violet	$1\cdot 10^{-10}$			
	Tetra-*n*-butylthiuram disulphide	Reddish violet	$2\cdot 10^{-10}$			
	Dipentamethylenethiuram tetrasulphide	Brown	$3\cdot 10^{-10}$			
	Tetramethylthiuram monosulphide	Dark brown	$4\cdot 10^{-10}$			
(8) Thiocarbonic acid derivatives	*(A) Xanthates*					
	Potassium methylxanthate	Bluish green	$4\cdot 10^{-10}$			
	Potassium ethylxanthate	Bluish green	$4\cdot 10^{-10}$	226 (3.95), 301 (4.25)	Water	6
	Potassium *n*-propylxanthate	Bluish green	$4\cdot 10^{-10}$			
	Potassium isopropylxanthate	Bluish green	$4\cdot 10^{-10}$	305 (4.2), 370 (1.7)	Water	3
	Potassium *n*-butylxanthate	Bluish green	$4\cdot 10^{-10}$			
	Potassium isobutylxanthate	Bluish green	$4\cdot 10^{-10}$			
	Potassium *n*-amylxanthate	Bluish green	$4\cdot 10^{-10}$	225 (3.9), 303 (4.2) 380 (1.7)	10% KOH	3
	(B) Others					
	Carbon disulphide***	Violet blue	[illegible]10^{-7}	314 (2.82), 319 (2.83)	Cyclohexane	10
(9) S-Sulphonic acids ($RSSO_3H$)	Pantetheine-S-sulphonic acid	Red	$5\cdot 10^{-8}$			
	4′-Phosphopantetheine-S-sulphonic acid	Red	$5\cdot 10^{-8}$			
	Sodium ethylthiosulphate			240 (1.4–1.66)		12
(10) Organothiophosphorus compounds	Parathion	Greyish violet	$5\cdot 10^{-10}$	275 (4.0), 340 (2.7)	Ethanol	5
	Fenthion (Baytex)	Red	$5\cdot 10^{-10}$			
	Ethion	Reddish violet	$1\cdot 10^{-9}$			
	Disyston	Reddish brown	$1\cdot 10^{-8}$			
(11) Thiocyanates (RSCN)	Methyl thiocyanate***	Red	[illegible]10^{-7}			
	Ethyl thiocyanate***	Red	[illegible]10^{-7}			

(12) Isothiocyanates (RNCS)	Methyl isothiocyanate***	Red	$\cdot 10^{-7}$			
	Ethyl isothiocyanate***	Red	$\cdot 10^{-7}$	245 (2.86)	Dioxane	10
	Cyclohexyl isothiocyanate***	Red	$1.5 \cdot 10^{-8}$			
(13) Sulphonic acids (RSO_3H)	Methanesulphonic acid	None	$> 10^{-7}$			
	Hydroxymethanesulphonic acid sodium salt	None	$\cdot 10^{-7}$			
	1-Butanesulphonic acid sodium salt	None	$\cdot 10^{-7}$			
	Cysteic acid monohydrate	None	$\cdot 10^{-7}$	Featureless 200–230	Water	4
	DL-Homocysteic acid	None	$\cdot 10^{-7}$			
	Taurine	None	$\cdot 10^{-7}$			
	D-Pantoyltaurine calcium salt	None	$\cdot 10^{-7}$			
	Sulphanilic acid	Red	$8.3 \cdot 10^{-11}$	248 (3.9), 290 (2.8)	Water	5
(14) Sulphates ($ROSO_3H$)	Dodecylsulphuric acid sodium salt	None	$\cdot 10^{-7}$			
	Hexadecylsulphuric acid sodium salt	None	$\cdot 10^{-7}$			
	D-Glucose-6-sulphate potassium salt	None	$\cdot 10^{-7}$			
(15) Sulphinic acids (RSO_2H)	Rongalite	None	$\cdot 10^{-7}$			
	Formamidinesulphinic acid	None	$\cdot 10^{-7}$			
(16) Sulphamic acid derivatives	Sulphamic acid	None	$\cdot 10^{-7}$			
	Ammonium sulphamate	None	$\cdot 10^{-7}$			
	Cyclohexylsulphamic acid sodium salt	None	$\cdot 10^{-7}$			
	Sulphanilamide	Red	$9 \cdot 10^{-11}$	262 (4.25)	Ethanol	4
	Sulphapyridine	Brown	$1.7 \cdot 10^{-11}$	245 (4.2), 263 (4.2), 312 (4.0)	Water	4
	N-Ethylethanesulphonamide			282 (0.7)	Dioxane	7
(17) Sulphones (RSO_2R')	Dimethyl sulphone	None	$\cdot 10^{-7}$	· 180		11
	Di-*n*-propyl sulphone	None	$\cdot 10^{-7}$			
	Di-*n*-butyl sulphone	None	$\cdot 10^{-7}$			
	Sulpholane	None	$\cdot 10^{-7}$			
	DL-Methionine sulphone	None	$\cdot 10^{-7}$			
	p,p'-Sulphonyldianiline	Purple	$1 \cdot 10^{-11}$			
(18) Sulphoxides ($RSOR'$)	Dimethyl sulphoxide	None	$\cdot 10^{-7}$	203 (3.4)	Cyclohexane	6
	DL-Methionine sulphoxide	None	$\cdot 10^{-7}$			
(19) Esters of inorganic acids and related compounds	Dimethyl sulphate	None	$\cdot 10^{-7}$			
	Dimethyl sulphite	None	$\cdot 10^{-7}$			
	Di-*n*-propyl sulphite	None	$\cdot 10^{-7}$			
	Ethyl methanesulphonate	None	$\cdot 10^{-7}$			

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* The colour was observed just after applying $1 \cdot 10^{-7}$ mole of the compound on a Wakogel FM plate.

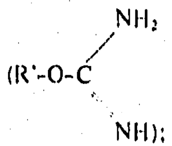
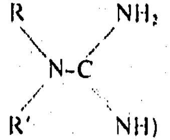
** Values after development.

*** The limits of detection of these compounds are not reproducible owing to their high volatilities.

TABLE II

LIMITS OF DETECTION OF NON-SULPHUR-CONTAINING ORGANIC COMPOUNDS ON THIN LAYERS CONTAINING MIXED FLUORESCENT MATERIAL

Classification	*Compound*	*Colour*	*Limit of detection (mole)*	*$\lambda_{max.}$ (log ε) (nm)*	*Solvent*	*Reference*
(1) Alcohols (ROH)	Methanol*	None	$\cdot 10^{-7}$	183,3 (2,18)	None	10
	Ethanol*	None	$\cdot 10^{-7}$	181 (2,51)	None	10
	n-Butanol*	None	$\cdot 10^{-7}$			
	1-Dodecanol	None	$\cdot 10^{-7}$			
	1-Octadecanol	None	$\cdot 10^{-7}$			
(2) Peroxides (ROOR')	Methyl peroxide			· 220	Hexane	13
(3) Ethers (ROR')	Dioxane*	None	$\cdot 10^{-7}$	180 (3,8)	None	4
	Diethyl ether*	None	$\cdot 10^{-7}$	171 (3,60), 188 (3,30)	None	10
	Diisoamyl ether*	None	$\cdot 10^{-7}$			
	Di-*n*-hexyl ether*	None	$\cdot 10^{-7}$			
	Di-*n*-octyl ether	None	$\cdot 10^{-7}$			
	Di-*n*-decyl ether	None	$\cdot 10^{-7}$			
	Di-*n*-dodecyl ether	None	$\cdot 10^{-7}$			
(4) Ureas (RR'NCONRR')	Urea	None	$\cdot 10^{-7}$	No maxima 220-250	0,1 *N* NaOH	4
	N,N'-Dicyclohexylurea	None	$\cdot 10^{-7}$			
	Semicarbazide hydrochloride	None	$\cdot 10^{-7}$	278 (· 0,7), 357 (· 1,1)	Water	4
	Barbituric acid	Red	$5 \cdot 10^{-10}$	257,5 (4,33)		4
	Barbital	Red	$6 \cdot 10^{-9}$	255 (2,9)	0,45 *N* NaOH	3

(5) Isourea and amidines	O-Methylisourea sulphate	None	$>10^{-7}$			
	Acetamidine hydrochloride	None	$>10^{-7}$	219 (3.04)		5
	Guanidine hydrochloride	None	$>10^{-7}$	262 (1.25)	Water	3
	Creatine	None	$>10^{-7}$	215 (3.08)		6
	L-Arginine	None	$>10^{-7}$	208 (2.52)		6
(6) Carboxylic acid derivatives	*(A) Carboxylic acids*					
	Acetic acid[*]	None	$>10^{-7}$	203	Water	4
	n-Caprylic acid	None	$>10^{-7}$			
	Palmitic acid	None	$>10^{-7}$	210 (1.70)	Ethanol	4
	L-Proline	None	$>10^{-7}$	Featureless 200–230	Water	4
	(B) Acid amides					
	Acetamide	None	$>10^{-7}$	220 (1.8)	Water	5
	Propionamide	None	$>10^{-7}$	175 (3.85)		3
	Formamide	None	$>10^{-7}$			
	N,N-Dimethylformamide	None	$>10^{-7}$	162.1 (3.84), 197.4 (3.94)	None	5
	(C) Lactones					
	γ-Butyrolactone	None	$>10^{-7}$	209 (1.63)	Methanol	10
	D-(—)-Pantoyl lactone	None	$>10^{-7}$			
	D-Glucuronolactone	None	$>10^{-7}$			
	(D) Esters					
	Ethyl acetate[*]	None	$>10^{-7}$	211 (1.76)	Ethanol	4
	2-Ethylbutyl acetate	None	$>10^{-7}$			
	2-Ethylhexyl acetate	None	$>10^{-7}$			
	(E) Acid anhydrides					
	Acetic anhydride	None	$>10^{-7}$	224.5 (1.68)	Isooctane	4

(Continued on p. 206)

TABLE II (*continued*)

Classification	*Compound*	*Colour*	*Limit of detection (mole)*	*λ*$_{max.}$ *(log ε) (nm)*	*Solvent*	*Reference*
(7) Amic acids ($RR'NCOOR''$)	Ammonium carbamate	None	$\cdot 10^{-7}$			
	Urethane	None	$\cdot 10^{-7}$	· 220	Water	14
	N-Methylurethane	None	$\cdot 10^{-7}$			
(8) Carbonates ($RO{-}\underset{\underset{O}{\parallel}}{C}{-}OR$)	Dimethyl carbonate*	None	$\cdot 10^{-7}$			
	Diethyl carbonate*	None	$\cdot 10^{-7}$			
(9) Organophosphorus compounds	Trimethyl phosphate*	None	$\cdot 10^{-7}$	229 (1.74), 262.5 (0.97)	Ethanol	5
	Trimethyl phosphite*	None	$\cdot 10^{-7}$	251 (2.76), 257 (2.80)	Ethanol	5
	Triethyl phosphite*	None	$\cdot 10^{-7}$	260 (0.16)	Ethanol	5
(10) Isocyanates ($RNCO$)	Ethylisocyanate	None	$\cdot 10^{-7}$			
	n-Butylisocyanate	None	$\cdot 10^{-7}$			
	Cyclohexylisocyanate	None	$\cdot 10^{-7}$			
(11) Ketones ($RCOR'$)	Acetone*	None	$\cdot 10^{-7}$	270 (1.25)	Water	4
	Methyl ethyl ketone	None	$\cdot 10^{-7}$			
	Cyclohexanone	None	$\cdot 10^{-7}$	285 (1.2)	Ethanol	5
	Diisobutyl ketone	None	$\cdot 10^{-7}$			
	2-Octanone	None	$\cdot 10^{-7}$	271 (1.41)	Ethanol	6
	2-Nonanone	None	$\cdot 10^{-7}$			
	2-Undecanone	None	$\cdot 10^{-7}$			
	3-Dodecanone	None	$\cdot 10^{-7}$			
	DL-Camphor	None	$\cdot 10^{-7}$	290 (1.48)	Ethanol	3
(12) Lactams	γ-Butyrolactam	None	$\cdot 10^{-7}$	190 (3.85)	Water	6
	δ-Valerolactam	None	$\cdot 10^{-7}$	195 (3.82)	Water	6
	ε-Caprolactam	None	$\cdot 10^{-7}$	197.5 (3.89)	Water	6
(13) Aldehydes ($RCHO$)	*n*-Capronaldehyde	None	$\cdot 10^{-7}$			
	n-Caprylaldehyde	None	$\cdot 10^{-7}$	295 (1.11)	Hexane	5
	n-Caprinaldehyde	None	$\cdot 10^{-7}$	222 (1.9), 293 (1.4)	Decane	4

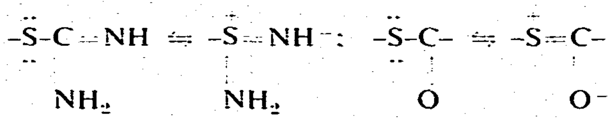
* The limits of detection of these compounds are not reproducible owing to their high volatilities.

colour. As can be seen from the results on disulphides in Table I, such characteristic colours were useful for the identification of different compounds.

As shown in Table III, the response of the mixed fluorescent material method to organic sulphur compounds is different from that reported with other methods. The fact that most biologically active organic sulphur compounds, including sulphonamides and pesticides, were detected by the present method indicates a promising establishment of a screen test for them, based upon a unique principle.

There are no other non-destructive methods for the detection of disulphides on paper or thin-layer chromatograms. In most instances of assay of disulphides, the sodium nitroprusside procedure[31] or the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) method[32] has been used after reduction of the disulphides with potassium cyanide or sodium borohydride. However, these methods are not specific for disulphides[33,34]. On the other hand, the detection of disulphides by the mixed fluorescent material method involves no destructive procedure unless the compounds in question are sensitive to UV light. Moreover, the limits of detection of simple disulphides are in the range 10^{-8}–10^{-9} mole, which indicates almost the same sensitivity as in conventional colorimetric methods[31,32]. The present method is now conveniently utilized in our laboratory in checking the synthetic reactions of mixed disulphides and also in the detection of lipophilic disulphides which are not easily subjected to cyanolysis.

The organic sulphur compounds designated as positive (detectable in amounts of less than $1\cdot10^{-7}$ mole) can be classified into four groups, according to their chemical structures. The first group contains thioureas (>N–CS–N<), thiuram compounds (>N–CS–S_n–CS–N<), xanthates (–O–CS–S–) and dithiocarbamates (>N–CS–S–), which contain at least one chromophore C=S group. The compounds belonging to the second group are disulphides (–SS–) and S-sulphonic acids (–SSO_3H), and their UV absorption at about 250 nm can be attributed to the S=S bond. The third group consists of isothioureas (–S–C(=NH)NH_2), thiolactones (S–CO–) and thioesters (–S–CO–) which can be represented in the resonanced C=S form as follows:



$$-\ddot{\underset{\cdot\cdot}{S}}-\underset{NH_2}{C}=NH \rightleftharpoons -\underset{NH_2}{\overset{+}{S}}=NH^{-}; \quad -\ddot{\underset{\cdot\cdot}{S}}-\underset{O}{C}- \rightleftharpoons -\overset{+}{S}=\underset{O^{-}}{C}-$$

The fourth group consists of organothiophosphorus compounds (>$\overset{|}{P}$=S). The presence of two pairs of lone-pair electrons on the sulphur atom (divalent sulphur) and a group that resonates with them is considered to be neccessary for the detection of compounds by the present method. This assumption is supported by considering the negative compounds, which can be classified into compounds that contain no divalent sulphur (RSO_3H, RSO_2H, $ROSO_3H$, RSO_2R' and RSOR') and compounds that contain a divalent sulphur atom but no resonating group (RSH and RSR').

Organic solvents that absorb UV light at 250–400 nm, *e.g.*, ketones and aromatic compounds, may interfere in the detection, but then volatile solvents such as benzene, acetone and carbon disulphide can be used. Alcohols, ethers, esters and hydrocarbons can be used without difficulty. In the analysis of compounds that have large R_F values, the use of plates developed once with the same solvent in order to

BLE III

RIOUS METHODS FOR THE CHROMATOGRAPHIC DETECTION OF SULPHUR COMPOUNDS

npound	*Present method*[2]	*I_2–NaN_3*[15,16]	*Iodo-platinate*[17,18]	*Chloro-platinic acid*[19,20]	*$HClO_4$–H_5IO_6*[21]	*KI–HCl*[22]
ols	−	+	+	+	+	−
ol acids	+					
phinic acids	−		+			+
phonic acids	−	−	−			−
phates	−					
phamates	−					
phenamides						
phinamides						
phonamides						
iocyanates	+					
thiocyanates	+					
ioketones	+					
ioamides	−					
ioureas	+	+		+	+	
thioureas	+					
ioesters	+					
iolactones	+					
iocarbamates	+					
thiocarbamates	+					
iurams	+					
inthates	+					
lphides	−	+	+	+		−
lphoxides	−		+	+		+
lphones	−		+	+		+
sulphides	+	+	+	+		−
iosulphinates						
iosulphonates						+
S'-Dioxides			+			
Sulphonic acids	−					−
iophosphorus compounds	−	−			+	
iophene	+				−	
iazole	−				−	
ilphur	+	−			−	
ilphide	+					
ilphite	−					
ilphate	−					
iosulphate	+	+				
hiocyanate	−	+			+	

remove traces of interfering substances in the adsorbent often increases the sensitivity.

The use of the present method prior to the application of spray reagents would markedly increase the reliability of detection.

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		+	+	+		+
				—		
	+			+		
				+		
				—		
		+				
		+	+			
		+	+	+		—
			+			—
						+
	+			+		
	+		+			
	+					
+		+	—	+		—
+				—		
+				—		
		+	—	+	+	—
				+		
				+		
	+					
					+	
			+			
					+	
					+	

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