CHROM. 15,060

THIN-LAYER CHROMATOGRAPHY WITH FLAME-IONIZATION DETECTION OF CHLOROMETHANESULPHONANILIDE AND ITS ETHOXYCARBONYLMETHYL DERIVATIVES

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(Received May 25th, 1982)

SUMMARY

A thin-layer chromatographic method with flame-ionization detection for the quantitative evaluation of mixtures from a reaction of chloromethanesulphonanilide with ethyl chloroacetate is described. The best results were obtained using Chromarods S II with *n*-hexane–dichloromethane–methanol (25:25:0.25 as the solvent system). The good accuracy and reproducibility in the range 2.5–100 μg suggest that this method could be of appreciable value for the analysis of compounds with chlorine and sulphur in the molecule, *e.g.*, for monitoring syntheses of pesticides.

INTRODUCTION

The combined use of thin-layer chromatography with a flame-ionization detection (TLC–FID) represents a significant advance as a method applicable to screening in biomedical and pharmaceutical areas[1,2]. This sensitive, relatively simple and rapid procedure also offers advantages in characterizing surfactants[3], fuel oils[4] and food additives[5], as well as for industrial research[6] analyses.

This paper reports our experiences with the TLC–FID technique in analysing products from the reaction of chloromethanesulphonanilide with ethyl chloroacetate. (Ethoxycarbonylmethyl)chloromethanesulphonanilide, appears to have a considerable application potential in herbicide preparations[7].

EXPERIMENTAL

Reagents and chemicals

Chloromethanesulphonanilide (CMSA) and N-(ethoxycarbonylmethyl)chloromethanesulphonanilide (Et-CMSA), prepared in our Institute, were at least 99% pure, melting points 74 and 51°C, respectively. Ethyl stearate (ES) (puriss.) was obtained from Fluka (Buchs, Switzerland).

All solvents were of reagent grade and were dried and distilled in glass before use.

Apparatus and conditions

The Iatroscan TH-10 Analyser, Mark II (Iatron Labs., Tokyo, Japan; distributed by Newman-Howells Assoc., Winchester, Hants., Great Britain) was connected to a Spectra-Physics Computing Integrator System I and a Linseis LS 24700 single-pen linear recorder.

Glass developing tanks (Iatron) were lined with Whatman No. 2 filter-paper which was pre-washed with mobile phase A or B (see below).

The stationary phase was Chromarod S II (Iatron) and the mobile phase were (A) *n*-hexane–benzene–methanol (40:10:0.5) and (B) *n*-hexane–dichloromethane–methanol (25:25:0.25). The scanning speed was 0.31 cm/sec (30-tooth gear), the chart speed 100 cm/min and the recorder range 100–800 mV full-scale.

Standard test mixtures

Seven standard solution mixtures were prepared for evaluation purposes. They included ethyl stearate as an internal standard and CMSA and Et-CMSA in various proportions (Table I).

TABLE I

COMPOSITION OF STANDARD SOLUTIONS

The concentration of ES was 5.0 μg/μl in all mixtures.

Mixture No.	Concentration (μg/μl)		Weight-%		
	Et-CMSA	CMSA	ES	Et-CMSA	CMSA
1	10.0	0.5	32.3	64.5	3.2
2	5.0	2.5	40.0	40.0	20.0
3	2.5	5.0	40.0	20.0	40.0
4	1.0	10.0	31.3	6.2	62.5
5	2.1	2.4	52.6	22.1	25.3
6	30.0	29.3	7.8	46.6	45.6
7	0.5	10.0	32.3	3.2	64.5

Procedure

A new set of Chromarods was twice blank-scanned in the FID burner and then immersed for 12 h in concentrated sulphuric acid. Prior to use, the rods were washed five times with *ca.* 200 ml of distilled water, dried for 10 min in a glass oven at 130°C and then subjected to a final single blank-scan. The activated Chromarods were then spotted with 1-μl portions of sample or standard solutions and developed for 30 min in one of the above-mentioned solvent mixtures. After oven-drying for 7 min at 100°C, the Chromarods were transferred to a scanning frame for detection.

Treated Chromarods whose low "noise" uptake was apparent or the separating capacity of which appeared to deteriorate (after *ca.* 5–7 analyses) were cleaned by immersion for 1 h in 6 N hydrochloric acid and by 5–6 subsequent washings with distilled water.

RESULTS AND DISCUSSION

Separation and R_F values

Of the many developing solvents previously tested, two appeared to be most suitable. As shown in Fig. 1a and b and in Table II, solvent B seems to be preferable because of a better baseline (lower FID noise) and good resolution of the three components in the central zone of the chromatogram, all of which provided a realistic basis for better reproducibility of analyses.
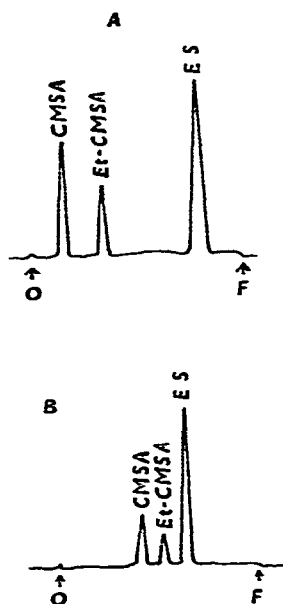

Fig. 1. Chromatogram of standard mixture No. 3. (A) Solvent system A; (B) solvent system B. Recorder range: 200 mV full-scale.

TABLE II

R_F VALUES OF Et-CMSA, CMSA AND THE INTERNAL STANDARD (ES) IN SOLVENT SYSTEMS A AND B

Solvent	ES	Et-CMSA	CMSA
A	0.81	0.35	0.16
B	0.65	0.52	0.42

Response of the FID and evaluation of the analyses

It is known in gas–liquid chromatography with an FID that the response of organic compounds depends on the proportion of carbon atoms that contain little or no oxygen[8]. This relationship need not be valid in TLC–FID, where the absolute weight response is a complex question involving both mechanical and chemical effects[1], e.g., the operating parameters of the detector[1,9], the relative volatility of the separated organic matter, the interaction between the organic material and adsor-

bent[10] and the shape and the position of the separated components on the Chromarod[8].

Nevertheless, under strictly controlled conditions, it may be assumed that FID responses are reproducibly proportional to the amounts of the components spotted. This relationship is presented in eqns. 1–3 from the analyses of standard mixtures (Table I) on seven different rods by means of the least-squares method using a digital computer:

$$A_{IS} = 18x_{IS} \ (r = 0.998) \tag{1}$$

$$A_{EA} = -9.6 + 12.1x_{EA} \ (r = 0.990) \tag{2}$$

$$A_A = -10.1 + 8.8x_A \ (r = 0.998) \tag{3}$$

where A_i is the relative area of a particular peak (the reading from the computing integrator $\times 10^{-3}$), x_i is the amount spotted, the subscripts EA and A correspond to Et-CMSA or CMSA, respectively, and r is the regression coefficient.

As can be seen, the characters of the calibration straight lines of the internal standard and of the two biocides are slightly different; the former line passes through the origin, whereas the latter two intersect the amount axis at 0.8 μg (Et-CMSA) and 1.1 μg (CMSA).

From the regression equations it can be concluded that $K_{FID(lim)}$ are about 1.5 for Et-CMSA and 2.1 for CMSA (K_{FID} of the internal standard $= 1.0$) (Fig. 2).
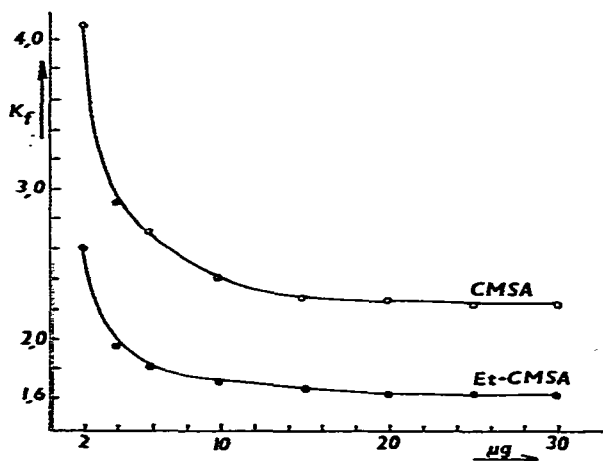

Fig. 2. Plot of K_{FID} *versus* amount spotted.

From a comparison with theoretical K_{FID} values, calculated from the molar percentages of carbon in the analysed components, one can deduce that the response of CMSA seems to be proportional to the content of aromatic carbons or, in other words, the carbon atoms that are bound to chlorine or sulphur do not contribute to the total response. However, the response of Et-CMSA is higher than that which corresponds to the content of total carbon atoms in the molecule (Table III).

TABLE III

THEORETICAL K_{FID} VALUES

K_{FID} = reciprocal response

Type of carbon	Et-CMSA*		CMSA*	
	A	B	A	B
All C	1.67	1.56	1.73	1.65
Aromatic C	1.95	1.71	2.02	1.92

* A, calculated from the percentage of the total carbon atoms in the internal standard (76.9%); B, calculated from the percentage of non-carbonyl carbon atoms in the internal standard (73.1%).

At this stage, no attempt has been made to provide an explanation for the different behaviour of the two compounds, which illustrates the complexity of FID responses in the TLC–FID technique.

From eqns. 1–3, the relationships for the concentration c_i (in mg/ml) or percentage P_i of a component i in the sample solution can be written as follows:

$$c_{EA} = \frac{x_{EA}c_{IS}}{x_{IS}} \tag{4}$$

$$c_A = \frac{x_A c_{IS}}{x_{IS}} \tag{5}$$

$$P_{EA} = \frac{x_{EA}}{x_{EA} + x_A} \cdot 100 \tag{6}$$

$$P_A = \frac{x_A}{x_{EA} + x_A} \cdot 100 \tag{7}$$

The values of c_{IS} and x_{IS} are known.

Accuracy and reproducibility

The accuracy of the method was checked by comparing theoretical values with averaged data, obtained from seven replicate analyses of standard mixtures 1–7 (see Table I). The results are summarized in Table IV.

Reproducibility was determined from the same experimental data by calculating the coefficients of variation (C.V.) (Table V).

It was shown that, within the range 2.5–30 µg, the experimental values are sufficiently accurate and differ from the theoretical values by only *ca.* 3%. At lower concentrations, however, the relative accuracy was strongly reduced and the corresponding deviations were, in some instances, greater than 100%, *i.e.*, the validity of the equations for Et-CMSA and CMSA is limited to values of x_i greater than 2.5 µg.

TABLE IV

COMPARISON OF THE THEORETICAL AND EXPERIMENTAL COMPOSITIONS OF STANDARD MIXTURES 1–7 (TABLE I)

Mixture No.	ES (wt.-%)		Et-CMSA (wt.-%)		CMSA (wt.-%)	
	Theoretical	Experimental	Theoretical	Experimental	Theoretical	Experimental
1	32.3	31.0	64.5	61.1	3.2	7.9
2	40.0	41.2	40.0	39.4	20.0	19.4
3	40.0	41.1	20.0	19.3	40.0	39.6
4	31.3	29.8	6.2	8.9	62.5	61.3
5	52.6	51.3	22.1	21.2	25.3	27.5
6	7.8	7.5	46.6	47.1	45.6	45.4
7	32.3	31.2	3.2	7.1	64.5	61.7

TABLE V

COEFFICIENTS OF VARIATON *VERSUS* THE AMOUNT OF Et-CMSA AND CMSA SPOTTED

Amount of ES spotted = 5 μg.

Mixture No.	ES C.V. (%)	Et-CMSA		CMSA	
		μg	C.V. (%)	μg	C.V. (%)
1	2.2	10.0	1.6	0.5	48.0
2	2.8	5.0	0.9	2.5	10.3
3	1.9	2.5	3.8	5.0	3.5
4	4.1	1.0	6.8	10.0	1.8
5	2.6	2.1	4.1	2.4	4.8
6	2.9	30.0	1.4	29.3	1.5
7	3.2	0.5	14.4	10.0	1.3

Similarly, the reproducibility of the calibration data also depends on the amount of substance analysed. In the concentration range below 2 μg it was poor (C.V. 10–50%); above 10 μg, however it was very good (C.V. *ca.* 1–2%), *i.e.*, comparable to, or even better than, that of acylglycerols and fatty acids[11].

The lower accuracy and reproducibility in the range 0.5–2 μg could be deduced from the non-linear dependence of the FID response on the loading of the rod. This behaviour could be caused not only by incorrect setting of the parameters of the computing integrator (*i.e.*, the preference of the small peaks which could be integrated together with part of the baseline), but also by the influence of elements other than carbon and hydrogen in the analysed material[1] and those in the thin layer.

The amount of sample accepted by the rod without any deterioration of separation and signal deformity may be influenced by many variables, including the separation ability of the Chromarod in a particular solvent system, the number of components in an analysed mixture and the chemical constitution of the separated compounds.

It was found advantageous to keep the amount of a single component spotted in the range 1–10 μg. Nevertheless, in mixtures containing minor components it might be necessary to operate with higher loads in order to obtain sufficient accuracy and reproducibility at small concentrations. Included in such instances are reaction mixtures of Et-CMSA and CMSA, especially those taken from the starting or final period of the synthesis.
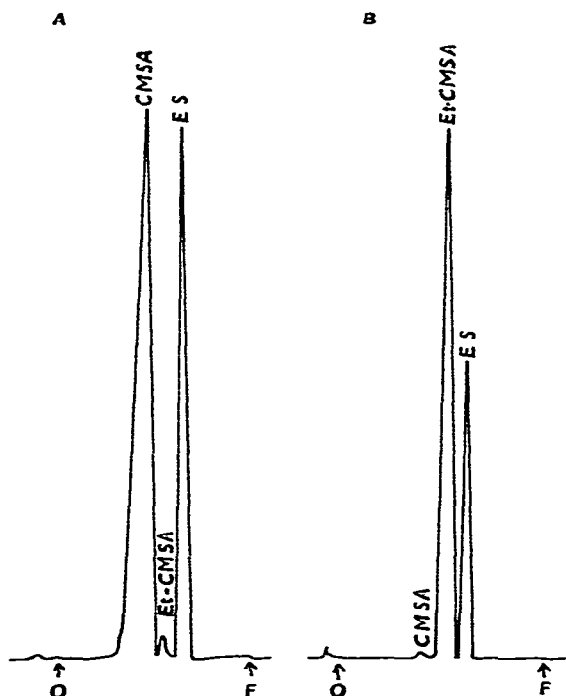

Fig. 3. TLC–FID analysis of the reaction mixtures. (A) Starting period of the reaction; (B) final period of the reaction. Solvent system B. Recorder range: 800 mV full-scale.

TABLE Vi

TLC–FID ANALYSES OF SAMPLES FROM THE REACTION OF CMSA WITH ETHYL CHLOROACETATE

Analysis No.	Starting period (wt.-%)			Final period (wt.-%)		
	ES	Et-CMSA	CMSA	ES	Et-CMSA	CMSA
1	17.07	1.66	81.27	19.08	78.93	1.99
2	17.48	1.57	80.95	19.78	78.38	1.84
3	16.56	1.32	82.12	19.76	78.20	2.04
4	17.54	1.53	80.93	19.18	78.80	2.02
5	17.74	1.39	80.87	20.34	77.55	2.11
6	17.30	1.47	81.23	20.12	77.90	1.98
7	17.42	1.63	80.95	19.37	78.41	2.22
Mean	17.30	1.51	81.18	19.66	78.31	2.03
C.V. (%)	2.5	8.3	0.6	2.4	0.6	6.9

The results of the analyses of two such mixtures are shown in Fig. 3 and Table VI.

In these analyses, each rod was spotted with *ca.* 120 μg of the dry substance, *i.e.*, with about 100 μg of the major and 2 μg of the minor component. In spite of such high loads, the separation of all the peaks was sufficient and the coefficient of variation of the minor component was well below 10 % and that of the major components even below 1 %.

TABLE VII

COMPARISON OF TRUE AND OBSERVED COMPOSITIONS OF STANDARD SOLUTIONS AT HIGHER LOADINGS

Average values of seven replicate determinations.

Mixture No.	Compound	Amount spotted (μg)	True composition (wt.-%)	Observed composition (wt.-%)	C.V. (%)	Absolute error (wt.-%)	Relative error (%)
8	ES	20.6	16.7	17.2	3.7	+0.5	2.9
	Et-CMSA	1.6	1.3	1.1	22.0	−0.2	15.4
	CMSA	101.5	82.0	81.7	0.6	−0.3	0.4
9	ES	19.9	16.5	16.9	1.8	+0.4	2.4
	Et-CMSA	98.0	81.0	80.8	0.5	−0.2	0.2
	CMSA	3.0	2.5	2.3	8.0	−0.2	8.0

To ascertain whether eqns. 1–3 are valid also at these high spotted amounts, two further standard solutions were prepared. The results given in Table VII are sufficiently precise and accurate to show the applicability of these relationships.

REFERENCES

1 R. G. Ackman, *Methods Enzymol.*, 72 (1981) 205.
2 M. Ranný, *Chem. Listy*, in press.
3 O. Cozzoli, *Riv. Ital. Sostanze Grasse*, 57 (1980) 136.
4 J. Newman, *Optimizing Reproducibility with Iastroscan TLC–FID Analyser, Fingerprinting of Heavy Oil by TLC–FID*, Instrument Application, Newman-Howells Assoc. Ltd., Winchester, Hants, 1981.
5 F. Bindler, P. Laugel and M. Hasselmann, *Deut. Lebensm.-Rundsch.*, 75 (1979) 111.
6 J. Poré, J. P. Houis and I. Rasori, *Rev. Fr. Corps. Gras.* 28 (1981) 111.
7 M. Zbirovský and V. Starý, *Sb. Vys. Sk. Chem.-Technol. Praze*, Org. Chem. Technol., C28, submitted for publication.
8 J. C. Sipos and R. G. Ackman, *J. Chromatogr. Sci.*, 16 (1978) 443.
9 T. Fujii, R. Tanabe, K. Sasai and T. Tanaka, *Yukagaku*, in press.
10 R. G. Ackman, *Chem. Ind. (London)*, (1981) 715.
11 M. Ranný, J. Sedláček, E. Mareš, J. Svoboda and R. Seifert, *Seifen, Fette, Oele, Wachse*, submitted for publication.