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COMPARATIVE STUDY OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY FOR THE DETERMINATION OF METOXURON AND ITS BY-PRODUCTS IN FORMULATIONS

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SUMMARY

Thin-layer and high-performance liquid chromatography were compared with respect to speed, sensitivity, reproducibility etc. for the determination of the urea herbicide metoxuron and some of its by-products in commercial formulations. Thin-layer chromatography was carried out on silica gel with chloroform–ethyl acetate (80:20, v/v), quantitation being effected by means of densitometry at 244 nm. High-performance liquid chromatography was performed on silica gel with dichloromethane–methanol–acetic acid (97.8:0.2:2, v/v), UV absorption at 244 nm being used for detection.

INTRODUCTION

Metoxuron [3-(3-chloro-4-methoxyphenyl)-1,1-dimethylurea] is a selective post-emergence herbicide and is commercially available as an 80% wettable powder.

Metoxuron formulations are usually analysed by titration of the dimethylamine, which is liberated via hydrolysis and distillation[1]. This method is not specific as most by-products will be included, and specific techniques therefore have to be coupled to chromatographic separations. As with many other phenylurea herbicides, the selective determination of trace amounts is a problem owing to the lack of sensitive methods. Gas chromatographic (GC) techniques, although possible[2,3], have to be carried out under difficult conditions if decomposition is to be avoided. As a consequence, some of the inherent sensitivity and convenience of GC are lost. On the other hand, both thin-layer and column chromatography are good alternative techniques for ureas.

For quantitative work in residue analysis, a fluorogenic labelling technique has been proposed[4]. A recommended technique for the analysis of metoxuron formulations involves thin-layer chromatographic (TLC) separation, elution of the

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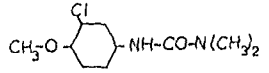
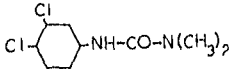
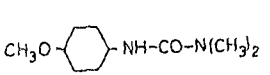
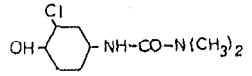
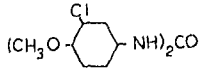
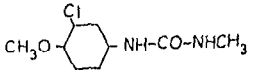
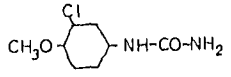
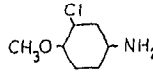
TABLE I

CHARACTERISTICS OF METOXURON AND SOME OF ITS DEGRADATION AND BY-PRODUCTS

Compound	*Systematic name*	*Structural formula*	*Abbreviation used in text*	λ_{max} *(nm)*	$\epsilon_{244\ nm}$ *(l·mole^{-1}·cm^{-1})*
Metoxuron	3-(3-Chloro-4-methoxyphenyl)-1,1-dimethylurea		—	244	18,000
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea		—	250	18,500
—	3-(4-Methoxyphenyl)-1,1-dimethylurea		4-Methoxy	242	17,300
—	3-(3-Chloro-4-hydroxyphenyl)-1,1-dimethylurea		3-Chloro	240	15,100
—	Bis-(3-chloro-4-methoxyphenyl)-urea		Bis	258	16,600
Monomethylmetoxuron	3-(3-Chloro-4-methoxyphenyl)-1-methylurea		MMM	244	25,100
Desmethylmetoxuron	3-Chloro-4-methoxyphenylurea		DMM	243	19,100
o-Chloro-*p*-anisidine	3-Chloro-4-methoxyaniline		*OCPA*	238	7700

metoxuron and UV measurement[5]. This permits the selective quantitation of metoxuron. Because this technique is not sensitive enough for by-products, they are determined semi-quantitatively by visual comparison on the plate. The use of densitometric techniques for the quantitation of TLC plates containing metoxuron and by-products seems feasible[6].

High-performance liquid chromatography (HPLC) is also a powerful technique for ureas, as has been demonstrated by Kirkland[7], and is also suitable for the analysis of metoxuron formulations[8]. The separation of metoxuron and its by-products, however, takes about 1 h, which is long for routine production control of large series of samples and causes considerable band broadening for the more polar compounds with a sometimes critical loss in sensitivity.

In this work, we have attempted to optimize the possibilities of TLC used in conjunction with densitometric techniques as well as the work of Sidwell and Ruzicka[8]. A critical comparison of TLC and HPLC and an earlier technique for Metoxuron formulations[5] has been made. The final aim was to develop techniques with good potential for trace residue analysis and metabolite work with metoxuron.

EXPERIMENTAL

Reagents

All solvents were of analytical-reagent grade purchased from Merck (Darmstadt, G.F.R.) or Baker (Phillipsburg, N.J., U.S.A.), and were used without further purification. The structures of the urea herbicides, obtained as gifts from Sandoz (Basle, Switzerland), are given in Table I.

Apparatus and procedures

TLC. Chromatography was carried out on 20 × 20 cm silica gel 60 F254 plates (Merck), which were eluted with acetone and air-dried before use. Twelve spots (four for standards and eight for samples) were applied to each plate, using a 10-μl micro-dispenser (Drummond, Broomall, Pa., U.S.A.). They were placed 15 mm from each other, 25 mm from the edge and 12 mm from the bottom, after which drying took place and pre-concentration with acetone up to 2 cm from the bottom.

For metoxuron the data-pair technique[9] was used. Three standard solutions and three samples were spotted in such a way as to give a pair for each concentration, with the two spots being about half a plate width apart. The average of four densitometric readings per spot (two forward and two backward scans in the direction of chromatography) was then taken.

The plates were placed in a 21.5 × 21.5 × 6 cm rectangular tank (Camag, Muttenz, Switzerland) in such a way that about 5 mm at the top of the plates protruded from the tank in order to promote evaporation of the mobile phase. The walls of the tank were covered with filter-paper to accelerate the saturation of the tank atmosphere. The plates were accommodated for 30 min and (over-)developed for 2 h (metoxuron) or 5 h (by-products), using chloroform–ethyl acetate (80:20, v/v) as the mobile phase. After development, the plates were air-dried and scanned at 244 nm, using a Zeiss PMQ II spectrophotometer (Zeiss, Oberkochen, G.F.R.) with a densitometer attachment and a Kipp BD-8 recorder (Kipp, Delft, The Netherlands). The reflectance was measured on the percentage transmission scale. The scanning speed

was 50 mm·min^{-1} and the width and height of the slit were 1 and 3 mm, respectively. Stock solutions of 10 μg·ml^{-1} for metoxuron and 1 mg·ml^{-1} for the by-products were prepared in either ethanol or dichloromethane–methanol (99.5:0.5, v/v), and were stored in a refrigerator.

HPLC. The liquid chromatograph was assembled from commercial parts. The pumping device was an Altex-100 pump (Altex, Berkeley, Calif., U.S.A.); the injection port was a Rheodyne six-port valve (Rheodyne, Berkeley, Calif., U.S.A.) with a 20-μl sample loop. Detection was effected by means of a Unicam LC-3 variable-wavelength UV detector (Pye Unicam, Cambridge, Great Britain) operated at 244 nm, the signal of which was recorded by means of a Servogor S recorder (Goerz, Vienna, Austria). The separation column was a 10 cm × 4.6 mm I.D. stainless-steel tube, pre-packed with 5-μm LiChrosorb SI-100 silica gel (Brownlee, Santa Clara, Calif., U.S.A.). Dichloromethane–methanol–acetic acid (97.8:0.2:2, v/v) was used as the mobile phase at a flow-rate of 1.0 ml·min^{-1}. All solvents were degassed before use. Stock solutions of metoxuron (0.1 mg·ml^{-1} in 0.5% methanol in dichloromethane) and the by-products (1 and 10 mg·ml^{-1} in 0.5% methanol in dichloromethane) were stored in a refrigerator.

UV spectroscopy. UV spectra were recorded on a Beckman Acta CIII spectrophotometer (Beckman, Fullerton, Calif., U.S.A.) using quartz cells with a path-length of 1 cm.

RESULTS AND DISCUSSION

Thin-layer chromatography

Separation. The solvent systems tested with regard to the optimal separation of the compounds listed in Table I were chloroform–ethyl acetate[5] and methanol–dichloromethane[8] in various proportions. The former system gave the best results and the composition chloroform–ethyl acetate (80:20, v/v), which was recommended by Wisson *et al.*[5], seems to be in an optimal region (Fig. 1). In an attempt to shorten the separation time, the methanol–dichloromethane system used by Sidwell and

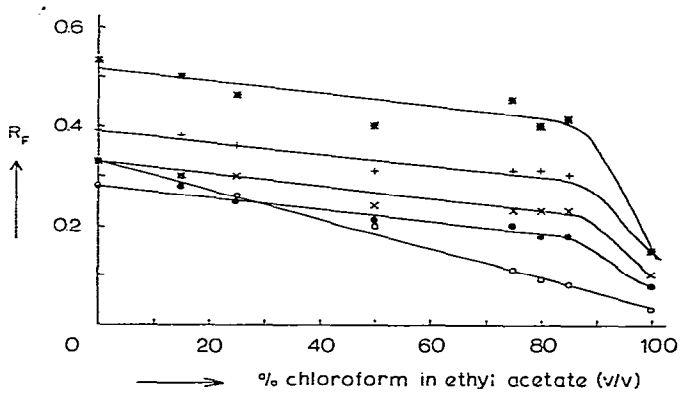


Fig. 1. R_F values for metoxuron and its by-products using chloroform–ethyl acetate mixtures as the mobile phase. Elution time, 10 min; temperature, ambient. * = Bis; + = diuron; × = metoxuron; ● = 4-methoxy; ○ = 3-chloro.

Ruzicka[8], who have used it for HPLC, was adopted. The same order of separation as found in HPLC was observed in TLC but the R_F values were too close together for a separation of all of the compounds, such as is needed for quantitative analysis, to be obtained. An attempt to improve this situation by introducing the solvent system methanol–tetrahydrofuran was unsuccessful.

Densitometry. The determination of metoxuron and its by-products was carried out by densitometry, using *in situ* diffuse reflectance scanning at 244 nm, which is close to the wavelength of maximum absorption for all of the compounds concerned (see Table I and Fig. 2). A typical densitometric scan is shown in Fig. 3.

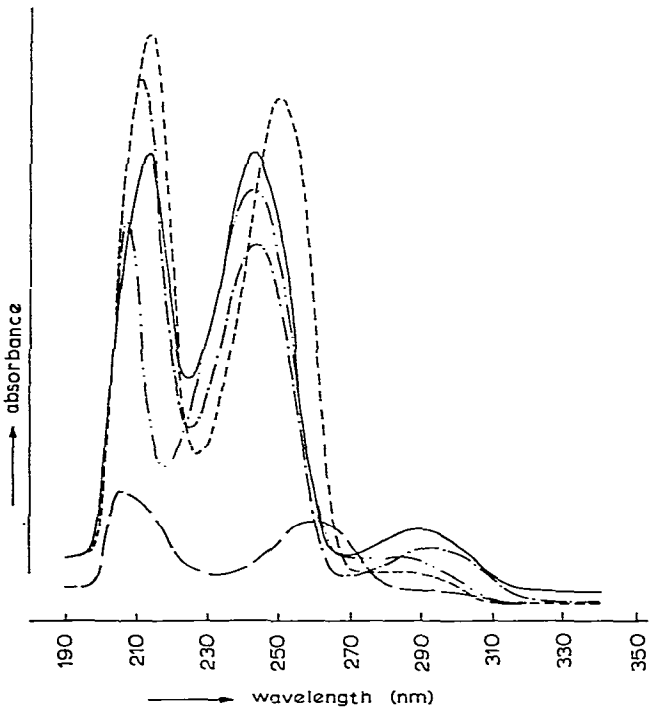


Fig. 2. UV absorption spectra of approximately 80 mg·ml^{-1} solutions of metoxuron and its by-products (except bis, 20 mg·ml^{-1}) in methanol. ——— = Metoxuron; — — — = diuron; —·· — = 4-methoxy; —·— = 3-chloro; —— = bis.

Calibration plots can be constructed by different techniques[6], of which the most common is a plot of the Kubelka–Munk function, $F(R_\infty)$, *versus* concentration, C. An adequate approximation is a plot of (peak area)^2 *versus* concentration. We used baseline-corrected peak-height measurements on the percentage transmission (% T) scale. For concentrations of 0.05–5 μg per spot linear log(100—T) *versus* log C plots were obtained and for 50–100 μg per spot linear log T *versus* log C plots were obtained. The method was tested with seven samples from different production batches. For the by-products the results, as an average of three determinations on each sample, are presented in Table II (see TLC-1 data). The detection limits for the by-products

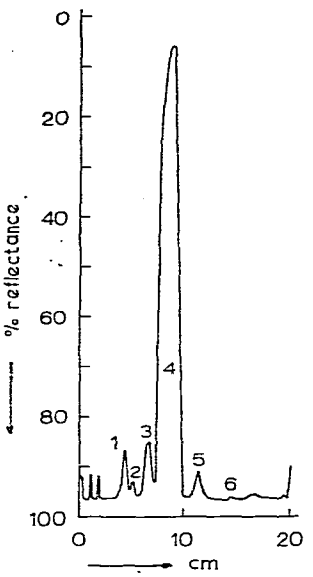


Fig. 3. Densitogram of industrial sample 7 of metoxuron (10 μg·ml^{-1}). Peaks: 1 = 3-chloro; 2 = MMM; 3 = 4-methoxy; 4 = metoxuron; 5 = diuron; 6 = bis. Conditions: pre-concentration with acetone; accommodation time, 80 min.

TABLE II

PERCENTAGES OF BY-PRODUCTS DETERMINED IN SEVEN INDUSTRIAL METOXURON SAMPLES

By-product	*Method**	*Sample No.*						
		1	*2*	*3*	*4*	*5*	*6*	*7*
Bis	HPLC	—	—	—	—	—	—	0.01
	TLC-1	—	—	—	—	—	—	0.01
	TLC-2	—	—	—	—	—	—	—
Diuron	HPLC	0.10	0.35	0.20	0.38	0.36	0.16	0.22
	TLC-1	0.10	0.38	0.16	0.39	0.38	0.14	0.20
	TLC-2	0.15	0.35	0.3	0.45	0.4	0.2	0.2
4-Methoxy	HPLC	0.10	0.14	0.08	0.13	0.15	0.08	0.51
	TLC-1	0.06	0.11	0.05	0.10	0.12	0.06	0.44
	TLC-2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.3
MMM	HPLC	—	0.022	0.007	0.026	0.03	0.012	0.09
	TLC-1	0.02	0.05	—	0.04	0.05	0.01	0.07
	TLC-2	—	—	—	—	—	—	—
3-Chloro	HPLC	0.006	0.012	0.015	0.015	0.015	0.005	0.28
	TLC-1	—	—	—	—	—	—	0.41
	TLC-2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	0.3

* HPLC and TLC-1, results of present study; TLC-2, data from ref. 5.

varied between 10 and 50 ng per spot. At concentrations above 0.1 % the by-products can be determined with a relative standard deviation better than $\pm$ 8 %.

For the determination of metoxuron itself, a separate chromatogram had to be developed using a 100-fold lower sample concentration for which only a 2-h sepa-

ration time was needed. The best results were obtained using the data-pair technique[9], which permits the simultaneous analysis of three samples together with three standards, both in duplicate. The regression coefficient was usually better than 0.999 and the reproducibility of the method was less than 1% (relative standard deviation) ($n = 4$). The average values of two separate determinations of metoxuron are given in Table III, together with data obtained by HPLC (see below) and by another technique[1,5].

TABLE III

PERCENTAGE OF METOXURON IN SEVEN INDUSTRIAL SAMPLES

*Sample No.**	*Method***		
	HPLC	*TLC-1*	*Hydrolysis*
1	101	100.8	99.5
2	99.7	97	98.9
3	99.6	99	99.1
4	99.1	97.4	99.4
5	100.3	97.3	99.3
6	99.2	99.6	99.4
7	97.6	97.4	98.8

* Sample numbers correspond to those in Table II.
** See footnote to Table II.

High-performance liquid chromatography

Separation. The first investigations, carried out with the binary mixture dichloromethane–methanol (99.5:0.5, v/v) yielded the separation shown in Fig. 4. The isocratic separation of the five components of interest is possible in principle on a column of length 10 cm and hence with a considerable time-saving in comparison with earlier results[8]. However, the 3-chloro component is so polar that it emerges from the column as a very flat, late-eluting peak ($t_R = 18$ min). The possibility of using a step gradient with higher methanol content for this last component is possible but introduces a complicating factor in routine control and a loss in time due to re-equilibration of the column for a re-run.

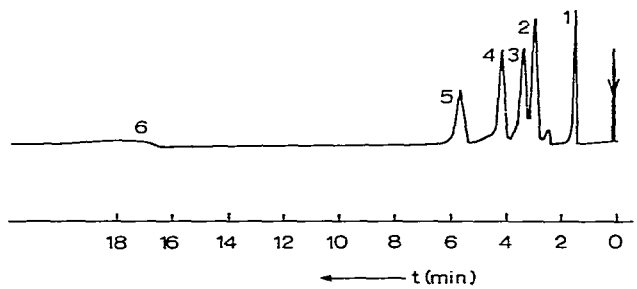


Fig. 4. HPLC separation of metoxuron and its by-products. Peaks: 1 = toluene; 2 = diuron; 3 = bis; 4 = metoxuron; 5 = 4-methoxy; 6 = 3-chloro. Mobile phase, dichloromethane–methanol (99.5:0.5, v/v); 0.32 a.u.f.s.

Adaptation of a ternary system consisting of dichloromethane–methanol–acetic acid resulted in a considerable improvement. The influence of the acetic acid concentration on the capacity ratio, k', is shown in Fig. 5. From this figure it appears that with 2% of acetic acid an optimal separation is possible. The order of separation of the pair diuron and bis is reversed and a better resolution is obtained (see Fig. 6).

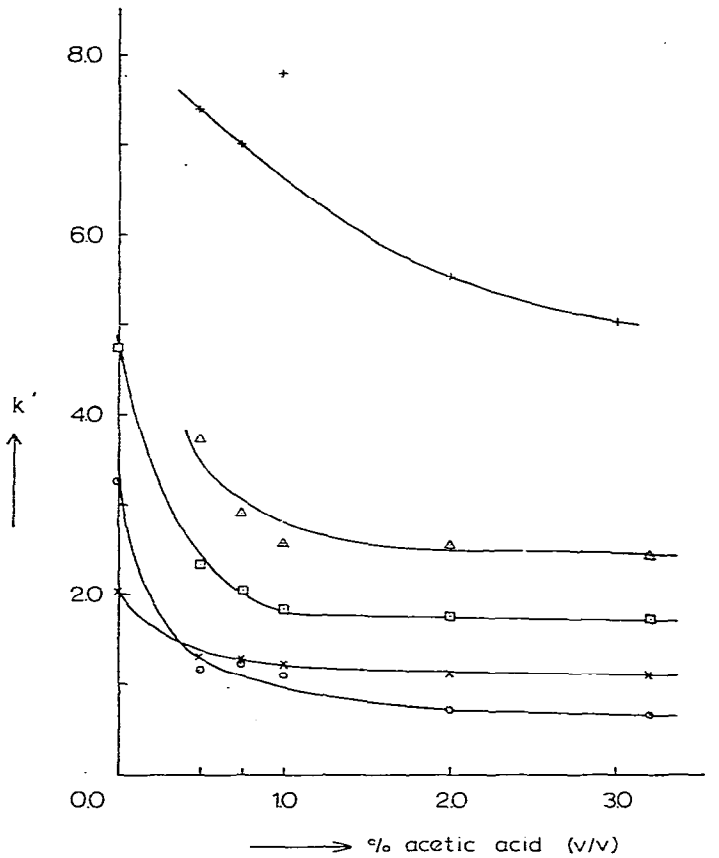


Fig. 5. Influence of composition of the mobile phase dichloromethane–methanol–acetic acid on k' values of metoxuron and its by-products. The methanol concentration was kept constant at 0.2% (v/v). + = 3-Chloro; △ = 4-methoxy; □ = metoxuron; × = diuron; ○ = bis.

Occasionally, one or two additional peaks appear in the chromatograms. One peak has been observed at $t_R = 5.5$ min ($k' = 3.3$) following the 4-methoxy peak. On the basis of retention data and standard additions it has been identified as monomethylmetoxuron (MMM). The nature of other peaks that precede the bis peak is unknown; one appears upon standing of the metoxuron solutions (see Figs. 7 and 8). The average k' values calculated from chromatograms run over a period of several months are 0.71 for bis, 1.10 for diuron, 1.75 for metoxuron, 2.52 for 4-methoxy and 5.5 for 3-chloro [relative standard deviation 4% < ($n = 50$) in all instances]. However, it must be noted that occasionally the separation efficiency of the column decreased.

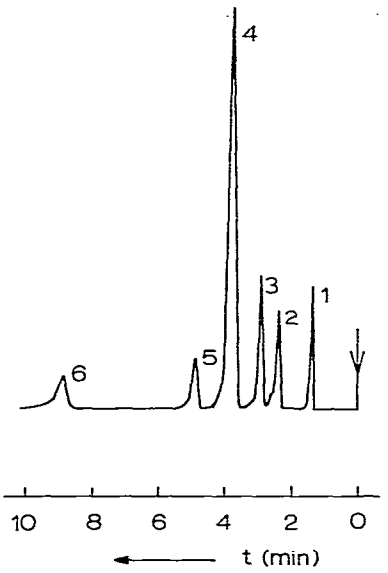


Fig. 6. HPLC separation of metoxuron and its by-products. Mobile phase, dichloromethane–methanol–acetic acid (97.8:0.2:2.0, v/v). Peaks: 1 = toluene; 2 = bis; 3 = diuron; 4 = metoxuron; 5 = 4-methoxy; 6 = 3-chloro.

In such cases reversing the column, *i.e.*, changing the injector and detector side, and flushing it with 30–40 ml of eluent considerably improved the situation and the plate number returned to its original value. If samples dissolved in ethanol are injected a drastic decrease in column performance is observed.

Quantitative analysis. Peak evaluation was carried out by peak-height measurements, using UV detection at 244 nm. Duplicate determinations were carried out for the by-products in seven samples. The calibration graphs were linear for all components in the concentration range 0.1–100 $\mu g \cdot ml^{-1}$ and passed through the origin. Sample solutions containing approximately 1 $mg \cdot ml^{-1}$ of metoxuron had to be prepared for the analysis of 4-methoxy and diuron, and 10 $mg \cdot ml^{-1}$ of metoxuron for 3-chloro, bis and MMM. Typical chromatograms are shown in Figs. 7 and 8. In order to determine metoxuron, quantitation was carried out using 100 $\mu g \cdot ml^{-1}$ sample solutions. All pertinent data are given in Tables II and III. For the by-products reasonable quantitation is possible even for the minor components ($< 0.1\%$). Relative standard deviations for the by-products are generally between 8 and 12% ($n = 7$), with the exception of diuron (1%); the detection limits are approximately 0.5 ng at a signal-to-noise ratio of 3. For metoxuron a relative standard deviation of 0.6% ($n = 7$) was obtained.

Comparison of results

A comparison of the results obtained by an HPLC and two different TLC techniques is possible from Tables II and III; the method corresponding to TLC-2 is that recommended by Wisson *et al.*[5]. There is generally good agreement between the techniques. Bis has only been found in sample 7 by our two techniques; this is not surprising, because the detection limit for TLC-2 is given as 0.2%. As for 3-chloro,

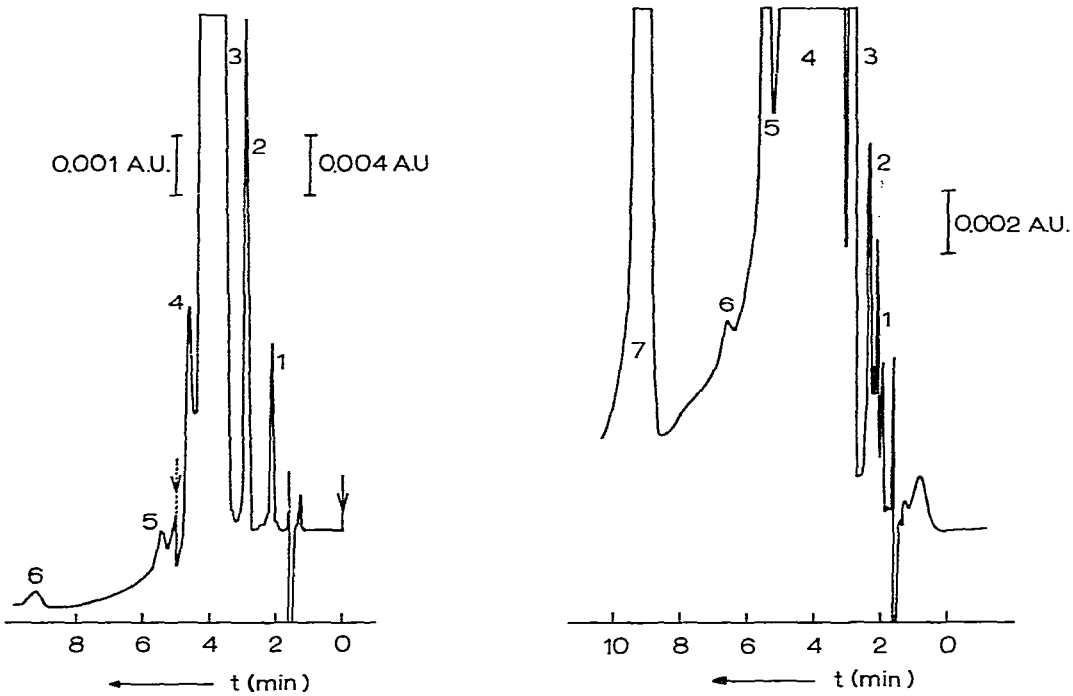


Fig. 7. HPLC separation of sample 2 [1 mg·ml^{-1} in dichloromethane–methanol (99.5:0.5, v/v)]. Peaks: 1 = unknown; 2 = diuron; 3 = metoxuron; 4 = 4-methoxy; 5 = MMM; 6 = 3-chloro. For conditions, see Fig. 6.

Fig. 8. HPLC separation of sample 7 (10 mg·ml^{-1} in dichloromethane–methanol (99.5:0.5, v/v). Peaks: 1 = unknown; 2 = bis; 3 = diuron; 4 = metoxuron; 5 = 4-methoxy; 6 = MMM; 7 = 3-chloro. For conditions, see Fig. 6.

because of its low level only HPLC yielded quantitative results in six out of the seven samples investigated. The presence of MMM, which is considered to be a degradation product of metoxuron, was not observed either by TLC-2 or by other workers[8]. Other known degradation products such as DMM and OCPA were not observed.

CONCLUSIONS

The two techniques developed in this work are adequate for formulation analysis. The HPLC method is more rapid, requiring 10 min per separation compared with 5 h (plus *ca.* 1 h scanning time) for TLC. One should bear in mind, however, that with the recommended data-pair technique[9] three samples can be analysed per plate. A reduction in the separation time is possible by using high-performance TLC (HPTLC). We have found that this technique can be used successfully employing the same chromatographic system. A more efficient separation is obtained in about 2 h (see Fig. 9); hence the time aspect becomes less important.

If large series of similar samples have to be analysed then HPLC offers the advantage of the possibility of automation. HPLC will also be the method of choice for residue analysis[10], because of its about 50-fold lower detection limit. For formu-

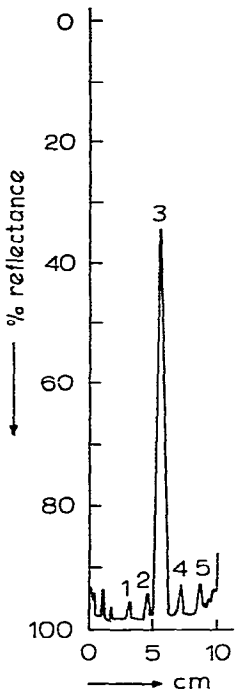


Fig. 9. Densitogram of metoxuron and by-products after elution on an HPTLC plate. Peaks: 1 = 3-chloro (0.12 $\mu g \cdot \mu l^{-1}$); 2 = 4-methoxy (0.107 $\mu g \cdot \mu l^{-1}$); 3 = metoxuron (9.37 $\mu g \cdot \mu l^{-1}$); 4 = diuron (0.090 $\mu g \cdot \mu l^{-1}$); 5 = bis (0.118 $\mu g \cdot \mu l^{-1}$). Conditions: 1-μl sample spots in ethanol; mobile phase, chloroform–ethyl acetate (80:20, v/v); elution time, 110 min; temperature, 23°; wavelength, 244 nm.

lation analysis, however, this is not so critical and one might prefer the TLC method with its inherent flexibility. From the point of view of economics, considering the cost of a densitometer, it would be about the same. The densitometer, however, can be shared by several working groups. If one is satisfied with semi-quantitative results for the by-products, the cheapest approach is the TLC technique proposed earlier[1,5]. Alternatively, metoxuron can be elegantly and reproducibly eluted from the TLC plate using an Eluchrom (Camag) and subsequently be determined by UV spectrometry. In our laboratory, for metoxuron and its degradation products (MMM, DMM and OCPA) standard graphs with regression coefficients of 0.998 in the range 0.8–8 μg per spot have been obtained.

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