Journal of Chromatography, 553 (1991) 489–496
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2102

Application of high-performance thin-layer chromatography and automated multiple development for the identification and determination of pesticides in water

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ABSTRACT

The combination of high-performance thin-layer chromatography (HPTLC) with automated multiple development (AMD) allows full automation of the separation step. This provides both a separation efficiency that is considerably better than that in conventional TLC and reproducible gradient elution on the thin layer. Reliable identification of trace amounts of pesticides (*e.g.*, 28 ppt of atrazine) in subsoil, surface and drinking water is clearly demonstrated by AMD-HPTLC using a polarity gradient based on dichloromethane. A second universal elution gradient with changed selectivity on which the substances have different relative migration distances, identification by the multi-wavelength response correlation and the option of recording UV spectra *in situ* constitute three independent methods for reliable and rapid verification. Using 100- instead of 200-μm HPTLC silica gel layers and reducing the running distance increments from 3 to 2 or 1 mm increase the sensitivity, the linearity and the speed of the method.

INTRODUCTION

The identification and determination of trace amounts of pesticides in subsoil, surface and drinking water is required by environmental protection legislation and is of great practical importance. The German drinking water guidelines have been adapted to those of the EC (European Community). They became effective on October 1st, 1986, and their practical implementation has proved a severe challenge to the supervisory authorities.

The tasks confronting these authorities become clearer when one considers the variety of substances to be determined and the low levels of active ingredients allowed per litre of water (0.1 μg for a single substance and 0.5 μg for the sum of all). A multi-sample method is very important, as usually a large number of samples containing many components have to be analysed. The requirements are met by the automated multiple development high-performance thin-layer chromatographic (AMD-HPTLC) method [1]. AMD does not require sample derivatization as do other methods such as gas and high-performance liquid chromatography.

0021-9673/91/$03.50

A multi-sample method of analysing multicomponent samples, using HPTLC and two polarity gradients for AMD, was described by Burger [1]. Burger [2], Weber [3,4] and Zietz and Ricker [5] used HPTLC silica gel plates, generally 200-μm thick and with 3-mm step increments for the polarity gradient. In a systematic investigation [6,7] using standard mixtures, a significant increase in the sensitivity of densitometric detection was obtained with AMD and thinner layers (100 μm).

This paper demonstrates the influence of reducing the layer thickness to 100 μm in combination with reducing the AMD step increments from 3 to 1 mm. The results of the chromatographic analysis of different samples with varying matrix contaminations and smaller amounts (ppt range) of active ingredients are presented. Reference is also made to the interdependence between the parameters described above and the linearity of the calibration graph. These parameters increase if the layer thickness and development step increments are reduced. To obtain an improved chromatographic resolution, the polarity of the first gradient [2,5,6] was optimized in accordance with ref. 5. The optimized method uses silica gel HPTLC plates of layer thickness 100 μm, running distance increments of 2 mm and a 25-step elution gradient containing ten isocratic steps at the start of chromatographic development.

EXPERIMENTAL

Reagents

The identification standards atrazine, desethylatrazine, desisopropylatrazine, isoproturon, terbutylazine, phenmedipham, metamitron, carbetamide, chloridazon, chlortoluron, carbofuran and metribuzin, all of ultra-pure quality, were obtained from Riedel-de Haën (purity at least 99%).

Ammonia solution (25%), concentrated hydrochloric acid, formic acid, acetonitrile (No. 30, gradient grade), *n*-hexane, *n*-heptane, *tert.*-butyl methyl ether (No. 1995 for residue analysis), methanol, dichloromethane and granulated activated carbon (No. 2518) of analytical-reagent grade were purchased from Merck.

Merck 60 F 254 s precoated HPTLC plates (20 × 10 cm) with a 200-μm standard layer thickness and plates from a test batch of the same material but with a 100-μm layer thickness (No. 11764) were used.

Solid-phase extraction glass cartridges, C_{18} (6 ml), silica gel (3 ml), were purchased from J. T. Baker.

It is essential that all reagents are of high quality and that the water is ultra-pure (deionized and membrane filtered).

Instruments

A Linomat IV, an AMD system, a TLC Scanner II with an IBM-compatible computer and CATS evaluation software, including multi-wavelength evaluation, were used. All instruments and software were supplied by Camag.

Sample preparation

C_{18} cartridges were conditioned by immersing them in 10 ml of acetonitrile and applying a vacuum to remove any air bubbles. They were then washed with 100 ml of ultra-pure water (adjusted to pH 2 with HCl). The cartridge was connected to a 1000-ml bottle containing the water sample (adjusted to pH 2 with HCl). The

connection was all glass (plastic tubing must not be used). The sample reservoir vent was connected to a small column packed with activated carbon. A flow-rate of 1–6 ml/min was maintained by means of a peristaltic pump connected to the cartridge outlet. A 1000-ml sample was percolated, then the cartridge was connected to a column packed with activated carbon and air was drawn through for 30 min by means of a water pump. The cartridge was then eluted with 3 ml of acetonitrile.

If the eluate from the C_{18} cartridge had a pronounced yellow colour (humic acid), it was percolated through a silica gel cartridge (1 ml) that had been conditioned with acetonitrile. This cartridge was eluted with 3 ml acetonitrile and washed with a further 1 ml of acetonitrile.

The eluate, either from the C_{18} cartridge direct, or the purified eluate from the silica column, was evaporated to dryness in a rotary evaporator at 35°C under nitrogen. The residue was dissolved in 0.2 ml of acetonitrile–*n*-heptane (95:5) and the solution obtained was used for chromatography.

Calibration and identification standards

All eleven pure substances (12.5 mg of each) were each dissolved in 25 ml of acetonitrile (11 stock solutions of 500 ng/μl). Standard mixtures were prepared as follows: 1 ml of an appropriate stock solution was added to and diluted to 20 ml with acetonitrile. A 1-μl volume of a mixture contains 25 ng of each compound.

The spring water was used as a sample for the determination of its content of atrazine and the metabolite desethylatrazine. The following two standard mixtures are routinely in use: mixture A, 1 ml of metamitron, 1 ml of carbetamide, 1 ml of chloridazon, 1 ml of chlortoluron, 1 ml of atrazine and 1 ml of carbofuran; and mixture B, 1 ml of desisopropylatrazine, 1 ml of desethylatrazine, 1 ml of isoproturon, 1 ml of terbutylazine and 1 ml of phenmedipham.

Preparation of spiked water samples and sample application

A 200-μl volume of each of the standard mixtures was added to 1 l of ultra-pure water, which corresponds to a 5 ppb level of each of the compounds present. These water samples were extracted as described under *Sample preparation*. HPTLC plates were prewashed by immersion for 1 h in isopropanol, followed by drying for 60 min at 120°C. Sample application was performed by the spray-on technique using a Linomat IV. Volumes of 10 μl each of the standard mixtures (A and B above), 10–80 μl of the extracts from the water samples and 10 μl of a blind extract (from non-spiked water) were applied as 8-mm bands, 4 mm apart; the delivery rate was 10 s/μl.

Chromatogram development

The chromatographic plates were developed using the AMD system with the 25-step universal gradient depicted in Fig. 1. This gradient, based on dichloromethane, is considered to be a screening gradient. In the event of a positive result, a second gradient based on *tert.*-butyl methyl ether is used for confirmation.

Prior to actual gradient development, the plate was developed for a distance of 8 mm with ten alkaline isocratic steps using pure acetonitrile, in order to move all soluble material away from the insoluble matrix at the area of sample application.

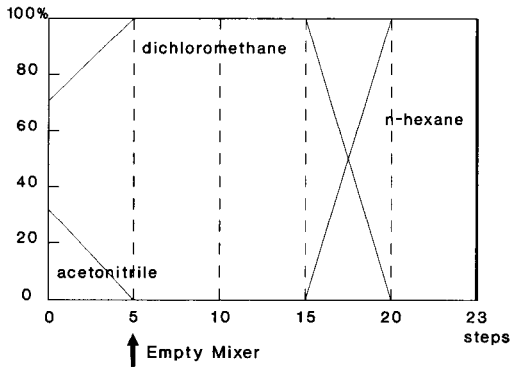


Fig. 1. The AMD elution gradient, designed for screening based on dichloromethane.

Densitometric evaluation

The underivatized plates were scanned by measuring the absorbance at six or seven different wavelengths in the TLC Scanner II using CATS evaluation software with the multi-wavelength option. The scanning parameters were a deuterium lamp, slit dimensions 0.2 × 5 mm, monochromator band width 10 nm and wavelengths 190, 200, 220, 240, 260, 280 and 300 nm; a multi-colour plotter was used for the graphical presentation of the analogue curves (coloured versions of Figs. 2–4 below are available on request). Using these scanning conditions and flushing the optical unit with nitrogen, each substance was quantified by the absorbance very close to its maximum response wavelength.

RESULTS AND DISCUSSION

The overall recoveries were determined on spiked, contaminant-free water and found to be 70% for metamitron, 79% for carbetamide, 95% for chloridazon, 88% for chortoluron, 98% for atrazine, 66% for carbofuran, 63% for desisopropylatrazine, 85% for desethylatrazine, 97% for isoproturon, 93% for terbutylazine and 92% for phenmedipham.

Fig. 2 shows multi-wavelength scans of the standard mixtures A and B. It can be seen that the identification standards are well resolved; they can be reliably identified by their multi-wavelength response correlation. An attempt to resolve all eleven substances on one track and identify them reliably was abandoned. Depending on the relative migration distances, many other combinations can be assembled into one group if screening for a certain selection is intended.

The analysis of a real sample of spring water is depicted in Fig. 3. Compounds identified were 72 ng/l (ppt) of atrazine (38.5 ng absolute), 94 ng/l of the metabolite desethylatrazine (50 ng absolute), 67.5 ng/l of chlortoluron (36 ng absolute) and 178 ng/l of 2,4-DP (dichlorprop) (95 ng absolute). These values were found with samples containing comparatively large amounts of humic acid. In the absence of humic acid, lower values can be expected.

The low-level detectability is shown in Fig. 4. The small peaks in the sample of drinking water are reliably identified as 28 ng/l of atrazine (7.5 ng absolute) and 43 ng/l of desethylatrazine (9.3 ng absolute).

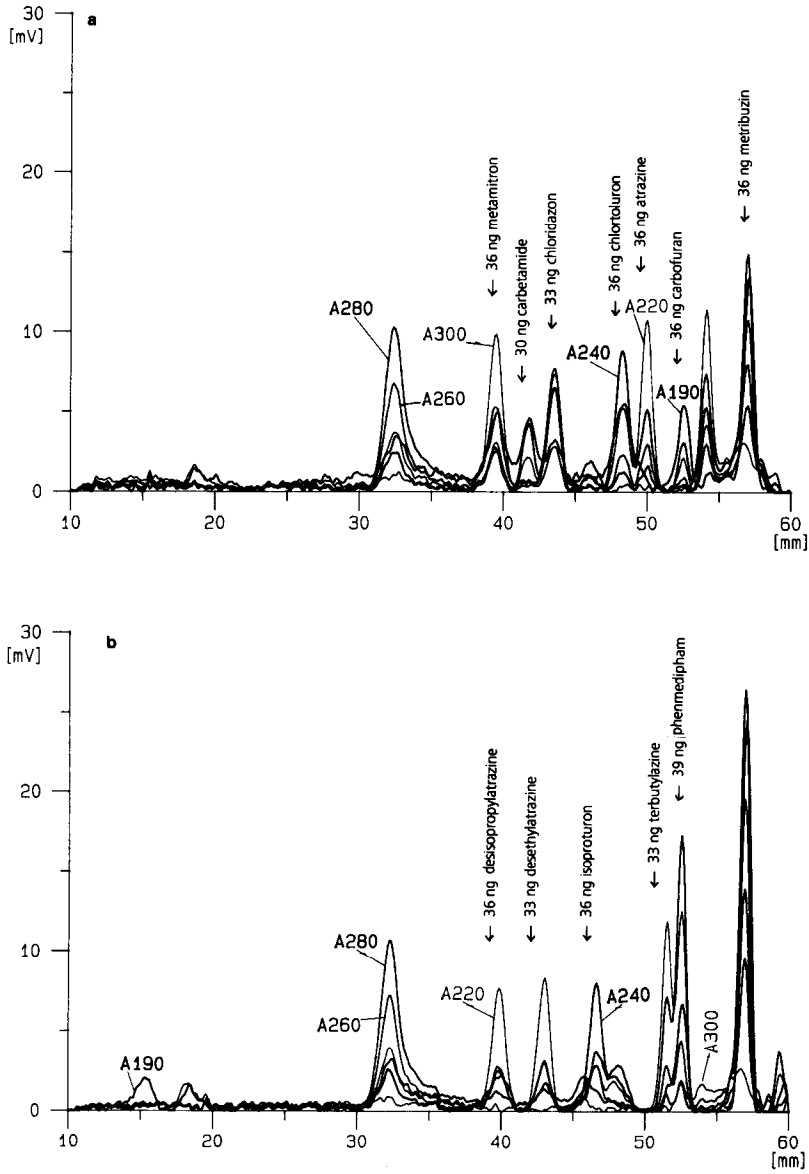


Fig. 2. Multi-wavelength scans of (a) standard mixture A and (b) standard mixture B, scanned at six different wavelengths (190–300 nm) with the plots superimposed. (Original, coloured plots of Figs. 2–4 are available from the authors upon request).

The influence of reducing the layer thickness from 200 to 100 μm on sensitivity is apparent from Table I and Fig. 5. The signals can be increased by a factor of 2.5 or more by reducing both the layer thickness and step increments. The linearity of the calibration graph and the dependence on the layer thickness and AMD step increments

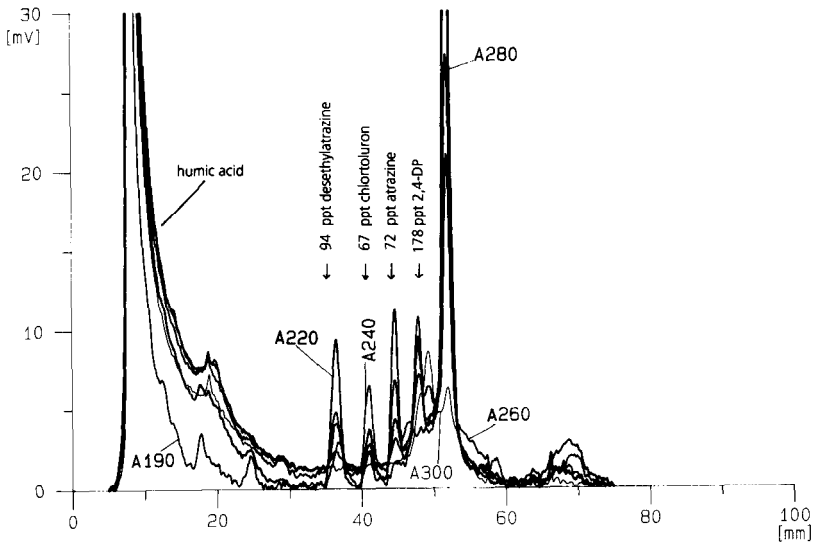


Fig. 3. Multi-wavelength scan of a spring water sample containing 72 ppt of atrazine, 94 ppt of desethylatrazine, 67 ppt of chlortoluron and 178 ppt of 2,4-DP (dichlorprop). The sample was scanned at six different wavelengths (190–300 nm) and the plots are superimposed.

are depicted in Fig. 6. Thinner layers and short step increments correlate with improved linearity of the peak-area (height) integrals *versus* concentration relationship.

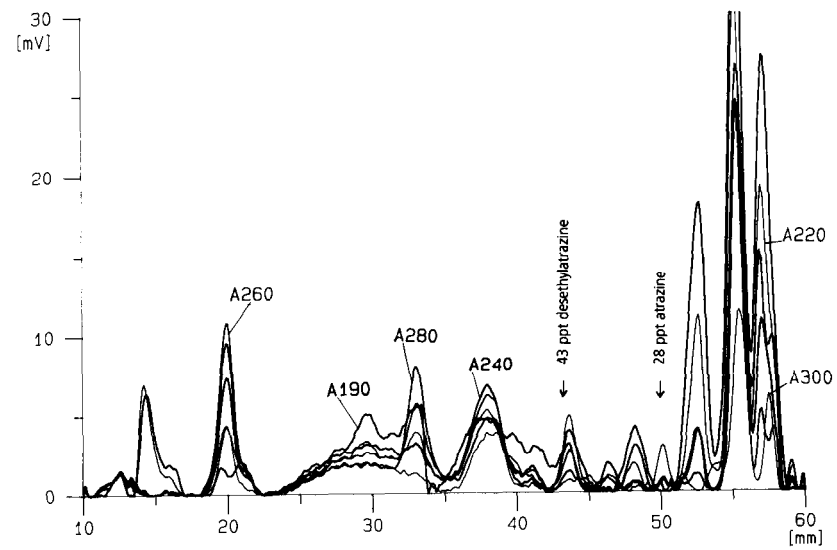


Fig. 4. Multi-wavelength scan of a drinking water sample containing 28 ppt of atrazine and 43 ppt of desethylatrazine.

TABLE I

RESPONSE AS ABSORBANCE AT 240 nm, MEASURED ON 200- AND 100-μm PLATES CHROMATOGRAPHED WITH DIFFERENT STEP INCREMENTS (1,2 and 3 mm)

The inconsistent ratios of the response under different conditions is believed to be due to the fact that some of the compounds had their absorption maximum away (up to 35 nm) from the measuring wavelength.

Component		Layer thickness (μm)					
		200			100		
		Step increment (mm)					
		3	2	1	3	2	1
Metoxuron	1	100	144	167	288	300	322
Monuron	2	100	133	167	267	292	308
Chlortoluron	3	100	125	158	250	267	275
Cyanazine	4	100	121	129	186	221	257
Propazine	5	100	84	100	142	179	179
Vinclozoline	6	100	82	75	163	138	150

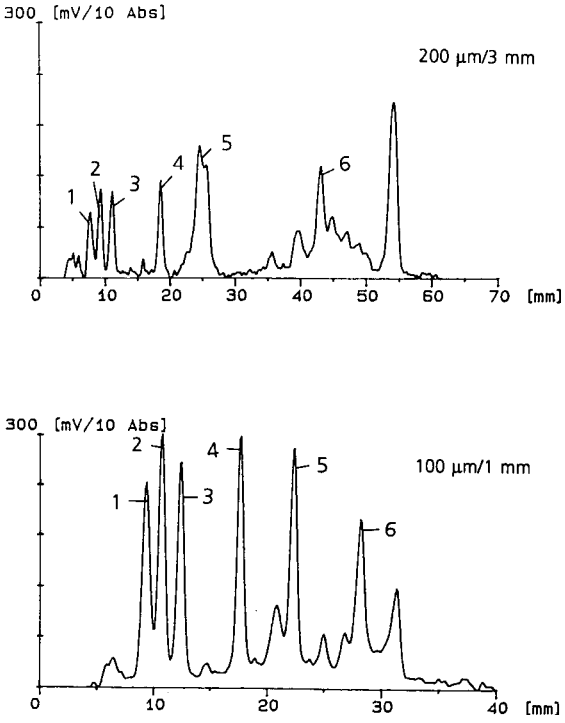
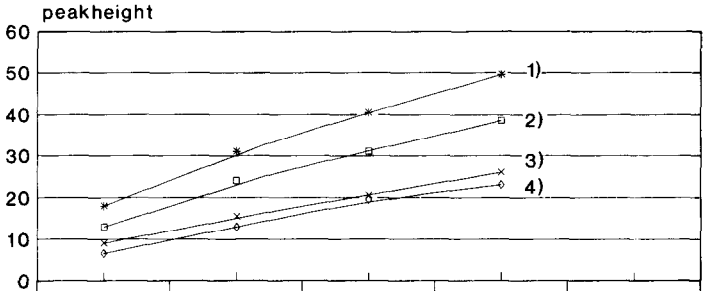


Fig. 5. Sensitivity and dependence on layer thickness of the mixture detailed in Table I.



		250 ng	500 ng	750 ng	1000 ng	Lin. Reg.
1)	100/1	18	31	40.5	49.5	y = 0.0408x + 9.0
2)	100/3	13	24	31	38.5	y = 0.0334x + 5.75
3)	200/1	9	15.5	20.5	26	y = 0.0224x + 3.75
4)	200/3	6.5	13	19.5	23	y = 0.0224x + 1.6

Fig. 6. Influence of the layer thickness and step increment of the AMD development gradient on the linearity of the calibration graph. 100/1, etc. = layer thickness (μm)/step increment (mm); the equations under Lin. Reg. are the calculated linear regressions of the calibration functions in the range of 250–1000 ng cyanazine; the values under 250–1000 ng are peak heights.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Birgit Knehr and Gerhard Keller of the Untersuchungs- und Forschungslabor Umwelt (Herbrechtingen, Germany) for valuable assistance with the samples.

REFERENCES

1 K. Burger, *Fresenius' Z. Anal. Chem.*, 318 (1984) 228.
2 K. Burger, *Pflanzenschutz-Nachr.*, 41 (1988) 172.
3 W. H. Weber, *Schriftenr. Inst. Wasser- Boden- Lufthyg. Bundesgesundheitsamtes*, 79 (1988) 183.
4 H. W. Weber, in F. Flegelmilch and W. David (Editors), *Tagungsband zur 3. Praktina (3. Europäisches Symposium für Instrumentelle Analytik), Krefeld, November 28–December 12, 1988*, Van Acken-Krefeld Verlag, 1988, p. 255.
5 E. Zietz, J. Ricker, *J. Planar Chromatogr.*, 2 (1989) 262.
6 H. W. Weber, presented at the *22. Hauptversammlung der Gesellschaft Deutscher Chemiker, Bonn, September 18–22, 1989.*
7 U. de la Vigne, D. E. Jänchen, *J. Planar Chromatogr.*, 3 (1990) 6.
8 *German DIN quota recommendation No. 38407 part 11. Determination of selected organic plant protecting agents by Automated Multiple Development (AMD) technique*, Deutsches Institut für Normung e.V., Berlin, in press.