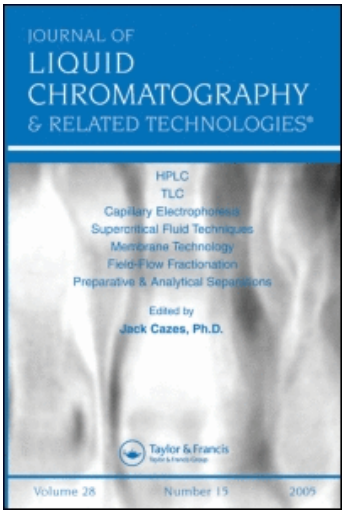

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DENSITOMETRIC QUANTITATION OF UREA, CARBAMATE, AND ANILIDE HERBICIDES ON C_{18} REVERSED PHASE THIN LAYERS USING BRATTON-MARSHALL DETECTION REAGENT

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ABSTRACT

Twelve urea, carbamate, and anilide herbicides were detected on KC_{18} reversed phase thin layers with the Bratton-Marshall reagent after hydrolysis to produce aromatic amines. Spots were quantitated at the nanogram level by scanning with a densitometer. Analyses of spiked water and soil samples were carried out to demonstrate the practicality of the method.

INTRODUCTION

Thin layer chromatography with densitometry has been widely used for the accurate and precise quantitation of residues of many classes of pesticides (1). The Bratton-Marshall detection reagent has been shown to be especially effective for determination of pesticides with an aniline moiety, such as asulam (2) and chloramben (3). The Bratton-Marshall reagent has also been used for the qualitative detection and semiquantitative (visual) determination of some substituted urea herbicides that are hydrolyzed to their anilines on thin layer plates (4). In this paper this approach has been extended to the detection of N-phenylcarbamate and anilide pesticides. Common hydrolysis conditions for these pesti-

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cides and quantitation of the resulting anilines by scanning densitometry are reported. The method is demonstrated with standard pesticides from these three classes and with soil and water samples fortified with representative pesticides.

EXPERIMENTAL

The following pesticide standards were obtained from the U.S.EPA pesticide repository, Research Triangle Park, NC: Urea herbicides-diuron (bromacil; 5-bromo-3-sec-butyl-6-methyluracil); linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea); monuron [3-(p-chlorophenyl)-1,1-dimethylurea]; neburon [1-n-butyl-3-(3,4-dichlorophenyl)-1-methylurea]; monolinuron [N-(4-chlorophenyl)-N'-methoxy-N-methylurea]; metrobromuron [3-(4-bromophenyl)-1-methoxy-1-methylurea]. Carbamates-barban (4-chloro-2-butynyl m-chlorocarbanilate); carbetamide [D-N-ethyllactamide carbanilate (ester)]; chlorpropham (isopropyl m-chlorocarbanilate); propham (isopropyl carbanilate). Anilide-propanil (3', 4'-dichlorophenylpropionanilide).

Stock solutions of these pesticides were prepared at the 10 mg/ml level in ethyl acetate, and these were volumetrically diluted to prepare other standard solutions, as required. Sample solutions were applied to layers with 1 µl Drummond microcap micropipets or a 25 µl Drummond Dialamatic microdispenser (1-25 µl variable delivery volume). Spiking solutions of metrobromuron and chlorpropham were prepared at a concentration of 0.50 mg/ml in ethyl acetate.

Whatman K5 silica gel and $KC_{18}D$ and $KC_{18}DF$ chemically bonded reversed phase thin layer plates (20x20 cm) were predeveloped with methanol-chloroform (or methylene chloride) (1:1 v/v) prior to spotting. Plates were developed in rectangular glass TLC tanks for a distance of 10-15 cm beyond the origin line, which was located

2.5 cm above the bottom of the plate. A pool of mobile phase 2-3 mm deep and a paper liner were contained in the tank, which was equiliberated for 10-15 minutes prior to inserting the spotted plate.

For detection of zones, the chromatogram was air dried and sprayed lightly and evenly, using a Kontes sprayer, with 6N ethanolic hydrochloric acid (60 ml of conc. HCl + 50 ml of absolute ethanol). The plate must not be oversprayed, or it will pucker; the layer should be wet but not visibly soaked. The sprayed layer was covered with a clean glass plate and placed in a 180°C oven for 10 minutes (5). The plate was removed from the oven and placed on an asbestos pad, and the layer was uncovered and allowed to cool. The layer was then sprayed with a 1% sodium nitrite solution until visibly saturated. This solution was prepared by dissolving 1.0 g of $NaNO_2$ in 20 ml of distilled water and diluting to 100 ml with 2N ethanolic HCl (17 ml conc. HCl + 83 ml ethanol). The layer was air dried (ca. 5 min) and then sprayed with a fresh 1% N-(1-napthyl)ethylenediamine dihydrochloride solution (1.0 g of reagent dissolved in 10 ml of H_2O, and diluted to 100 ml with ethanol) until visibly saturated.

Zones were scanned with a Kontes Model 800 fiber optics densitometer using the white phosphor (440 nm peak, 300 nm band width) and the single beam, transmission mode (BC setting) with an 8 mm light source length to match the 8 mm wide lanes of the KC_{18} divided plates. The scan rate was 5 cm/min. Recorder peaks were measured using the formula height x width at half-height.

Control pond water and soil (Keyport silt loam), which were pre-analyzed to prove they contained none of the herbicides of interest, were fortified by adding an appropriate volume of pesticide standard solution to the samples, which were shaken to distribute the spike.

Samples were prepared for TLC by procedures described by Ambrus et al. (6). Soil (100g) was extracted by shaking in turn with 100 ml and 70 ml of acetone containing 2 ml of 2N ammonium acetate. The extracts were filtered, combined, evaporated with nitrogen gas, and taken up in 4 ml of benzene. The benzene solution was cleaned up on a 1 cm id glass chromatography column containing 8 g of deactivated Woelm 200 neutral alumina (81 g Super I alumina + 19 g H_2O, mixed, and equiliberated for 2 hr). One ml of benzene solution was added to the column, which was pre-wet with 10 ml of hexane. The column was eluted with 30 ml of n-hexane (Fraction 1) and 30 ml of hexane-ethyl ether (7:3 v/v) (Fraction II). Lake water (1 liter) was extracted with 100, 50, and 50 ml portions of methylene chloride after adding 50 ml of saturated NaCl solution. The combined extracts were then treated as above for the soil extract.

Adsorbent was pretested with standard solutions of pesticides to assure proper elution characteristics of columns prior to the analyses. Column eluates were evaporated and the residue dissolved in ethyl acetate in a small tube to prepare the TLC spotting solution.

RESULTS AND DISCUSSION

Numerous mobile phases from the literature were tested for separation of the 12 pesticides of interest on silica gel thin layers. These included 2-, 3-, and 4-component solvents composed of various proportions of hexane, acetone, chloroform, and methanol; chloroform-nitromethane (1:3 v/v); and ethyl ether-toluene (1:3 v/v). Both saturated and unsaturated development conditions were evaluated. No system could be found that provided adequate resolution of the pesticides by silica gel TLC.

KC_{18} reversed phase plates with and without fluorescent indicator were then tested. Only the layers with

TABLE 1

R_F Values and Spot Colors of Pesticides on $KC_{18}F$ Layers Developed with Methanol-Acetonitrile-Tetrahydrofuran-Water (50:15:8:27 v/v)

Pesticide	R_F	Color
Diuron	0.47	red-purple
Fluometuron	0.55	red
Linuron	0.38	red-purple
Metobromuron	0.50	purple
Monolinuron	0.53	purple
Monuron	0.62	purple
Neburon	0.27	red-purple
Barban	0.33	red-purple
Carbetamide	0.60	red-purple
Chlorpropham	0.32	red-purple
Propham	0.49	purple
Propanil	0.40	red-purple

fluorescent indicator allowed detection of the zones; plates without fluorescent indicator did not show any spots. The presence of the phosphor apparently was necessary for successful completion of either the hydrolysis reaction or the detection reaction, or both.

Mobile phases for KC_{18} TLC were systematically designed by the procedure described by Sherma and Charvat (7) based on the paper of Lehrer (8). A solvent strength of 2.1 gave the optimum R_F range for the pesticides, and the mixture methanol-acetonitrile-tetrahydrofuran-water (50:15:8:27 v/v) was the best mobile phase with this strength. R_F values and spot colors in this system are shown in Table 1. Differences among R_F values would allow this method to detect and quantitate multiresidues of certain pesticides which are adequately re-

solved. This would be true for the three phenylcarbamates and for neburon, diuron, and monuron, for example. Developed zones were typically narrow and round in this system, generally covering an R_F range of ± 0.03 on either side of the R_F values in Table 1. All R_F values were within the optimum R_F range of 0.2-0.8 for accurate and precise densitometry (9).

As seen in Table 1, urea, phenylcarbamate, and anilide pesticides were detected as red, purple, or purple-red spots after in situ hydrolysis with ethanolic HCl, diazotization with $NaNO_2$, and coupling with Bratton-Marshall reagent. The reagent solution should be prepared fresh just before use and degrades within four hours. Spots appear immediately, and reach maximum color intensity within about 15 minutes after drying. The plate background becomes irreversibly purple after about 30 minutes, which allows more than enough time for scanning of the zones.

Representative compounds were chosen from each class of pesticides to demonstrate quantitation. These were metobromuron, monuron, and neburon (ureas), chlorpropham (carbamate), and propanil (anilide). Stock solutions of these compounds were diluted with ethyl acetate to prepare a series of standard solutions ranging in concentration from 10 ng/µl to 5 µg/µl. Increasing weights of each compound were spotted across individual plates, which were developed and the spots detected. For each of the five compounds, 50 ng was the lowest amount that was visually detected and 100 ng was the lowest amount that consistently gave a densitometer peak that was at least 10% of full scale deflection (10 times the noise level).

Peak areas (cm^2) were plotted vs ng spotted to construct calibration curves for each compound. Figure 1 shows the plot for 100-2000 ng of metobromuron. It is

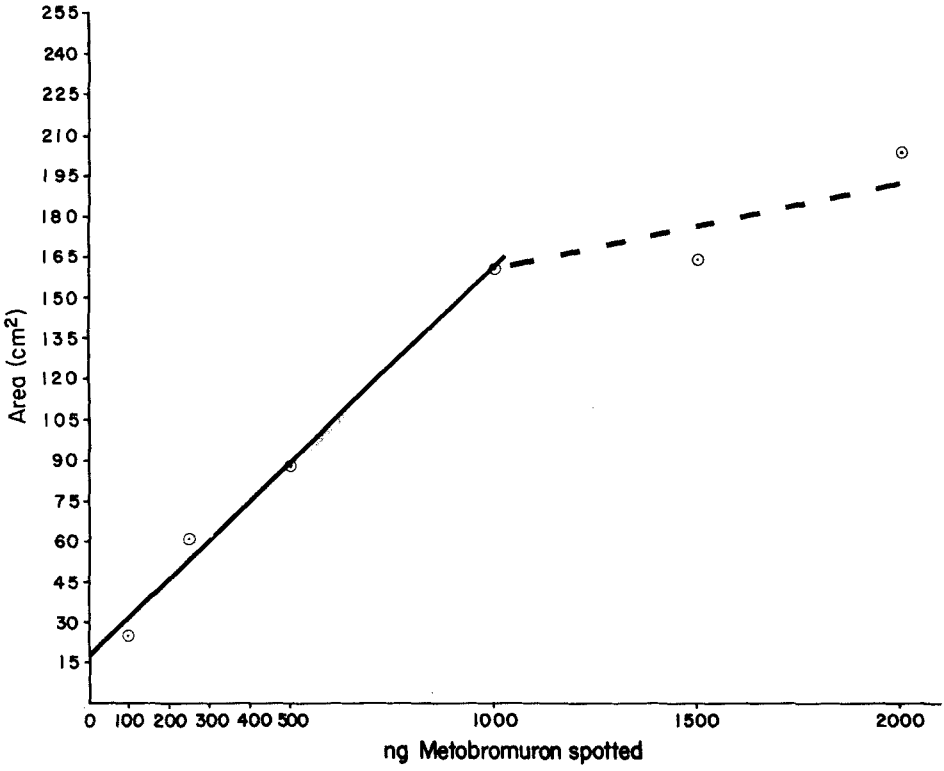

Figure 1. Calibration curve for the urea herbicide metobromuron from 100-2000 ng. Attenuation was X4 for the 100 ng peak and X8 for the others.

seen that the plot was linear from 100-1000 ng, and then curved downward toward the X-axis. Figure 2 shows the corresponding scanner peaks up to 1000 ng. The calibration plot for propanil was linear up to 1500 ng, monuron and neburon to 2000 ng, and chlorpropham to 3000 ng.

Calibration curves were quite reproducible in terms of slope, intercept, and linearity from plate to plate, but standards should always be run on the same plate with samples to obviate the effects of any variations when using the method.

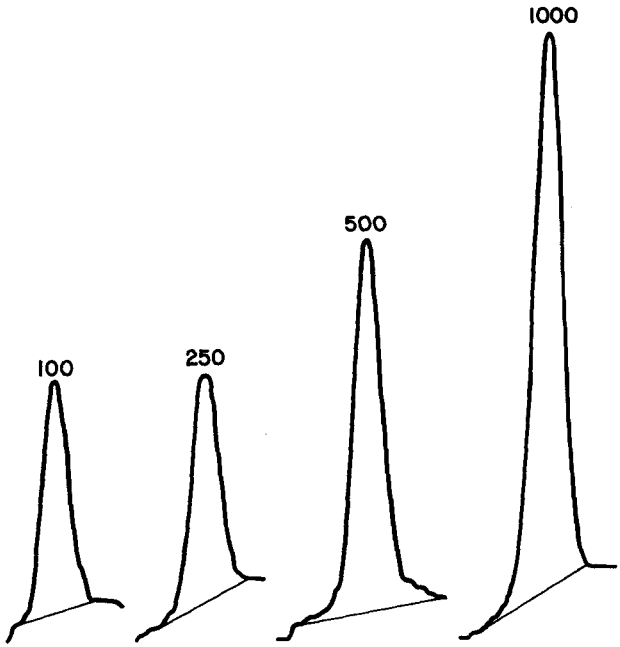

Figure 2. Scans of 100-1000 ng of metobromuron using the Kontes Model 800 scanner with attenuation 32. Constructed baselines are shown.

To check reproducibility, four 700 ng spots of each of the five compounds were spotted across separate KC_{18} plates, the plates were developed, and the zones were detected and scanned. The relative standard deviations of the areas ranged from 4.9 to 14.6%, with a mean of 9.8%. This is satisfactory precision considering the three spraying operations required for detection, and is more than adequate for pesticide residue determinations at concentrations below 1 ppm.

Lake water was spiked with chlorpropham and soil with metobromuron at 0.50 ppm by adding 500 µg of chlorpropham (10 ml of spiking solution) to 1.00 liter of control lake water and 50 µg of metobromuron (1 ml of spiking solution) to 100 g of air dried soil. Samples

were extracted and cleaned up by chromatography on a neutral alumina column as described in the Experimental section. Assuming complete recovery, the 1 ml of benzene solution added to the column represented 125 µg of chlorpropham (250 ml of water sample) and 12.5 µg of metobromuron (25 g of soil). The column eluate residues were dissolved in 200 µl of ethyl acetate, and 1 µl of the chlorpropham and 10 µl of the metobromuron sample solutions were spotted along with standards for TLC. If recovery was complete, these volumes would contain 625 ng of the respective pesticides. The 10 µl sample was spotted in small increments, with intermediate drying with a stream of nitrogen gas, to keep the initial zones compact and comparable in size to the standard zones. Duplicate samples and bracketing standards were applied to each plate, e.g., 200, 400, 600, 800, and 1000 ng.

The first (n-hexane) column eluate contained all of the chlorpropham, as reported by Ambrus et al (6). This was checked by spotting both eluates separately, which proved the second fraction was negative. Metobromuron split between the two fractions, with most of the pesticide being eluted in the first (6).

The recoveries reported by Ambrus et al. (6) for the column chromatography were greater than 80% for chlorpropham and 95% for metobromuron. Our overall recoveries from the spiked samples, obtained by interpolating the amount of pesticide in sample spots from the standard curve and comparing to the theoretical 650 µg level, averaged 78.6% (4 trials, range 74.0-83.3%) for chlorpropham in water and 81.8 (5 trials, range 76.5-84.8%) for metobromuron in water. Chromatograms had no zones interfering with the scanning of the pesticide zones. The chromatogram of the water sample was especially clean, and this sample could probably have been analyzed without column cleanup. The soil chromatogram had detected

zones at the origin plus one or two other zones well resolved from the pesticide.

SUMMARY

Advantages of TLC for pesticide analysis include simplicity, high sample throughput, and the ability to analyze multiple samples at the same time under identical conditions and to process standards in parallel. The method described in this paper is the first multiresidue procedure reported for determining urea, carbamate, and anilide type herbicides after detection as colored zones. Precision and accuracy (recovery) are shown to be adequate for analyses below 1 ppm, and two typical applications are demonstrated. Extension of the method to other sample matrices and additional pesticides of these types should be possible if the required extraction and cleanup steps are carried out.

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