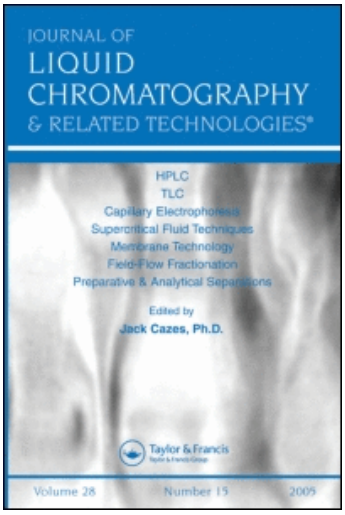

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Quantitative Analysis of Alachlor and Atrazine in Polymeric Microcapsules Determined by Reverse-Phase High Performance Thin Layer Chromatog-Raphy with Densitometry

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QUANTITATIVE ANALYSIS OF ALACHLOR AND ATRAZINE IN POLYMERIC MICROCAPSULES DETERMINED BY REVERSE-PHASE HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY WITH DENSITOMETRY

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ABSTRACT

A method to analyze polymeric microcapsules of the herbicides alachlor and atrazine by reverse-phase high performance thin layer chromatography (RP-HPTLC) has been developed. The herbicidal concentration is determined by densitometry. The method is rapid and reproducible and offers a practical alternative to determination of alachlor and atrazine by elemental analyses. Impurities, metabolites, and decomposition products which may yield falsely high percentages of herbicidal content as determined by elemental analyses are readily detected by RP-HPTLC and are not sources of error.

INTRODUCTION

Recently, concern over the pesticide contamination of groundwater has mounted. Selected pesticides have been detected at extremely low levels in groundwater in isolated

locations across the United States. In 1986, the U. S. Environmental Protection Agency disclosed that at least 17 pesticides used in agriculture had been found in groundwater in 23 states (1). According to a 1988 interim report, 74 different pesticides have been detected in the groundwater of 38 states from all sources. Contamination attributable to normal agricultural use has been confirmed for 46 different pesticides detected in 26 states (2).

The chief objectives of our research are to develop pesticide formulations that will maintain or increase efficacy against target organisms and that will not adversely impact on the groundwater. Microencapsulation is one method for obtaining this goal (3, 4). Microencapsulated pesticides should be safer to handle, exhibit controlled-release properties (thus possibly reducing the total amount of pesticide used), and have reduced potential for leaching in the soil profile while maintaining effective biological activity.

The herbicides alachlor and atrazine frequently have been implicated in groundwater contamination (1, 2). Previously, we reported the preparation of polymeric microcapsules of atrazine and the evaluation of their efficacy as herbicides under greenhouse conditions (5). Polymeric microcapsules of alachlor have also been prepared and evaluated in the greenhouse (O. D. Dailey, Jr. and C. C. Dowler, unpublished results).

In our continuing evaluation of polymeric microcapsules of atrazine and alachlor for long-term stability, volatility, and leaching properties, we sought an inexpensive, rapid, accurate, and reproducible method for determination of herbicidal content. In the past, herbicidal content was determined by the relatively expensive elemental analysis performed by a commercial laboratory. Often the percentage active ingredient determined

from N microanalysis did not agree with that obtained from Cl microanalysis, and resubmittal of samples was necessary. We have investigated high performance thin layer chromatography (HPTLC) with densitometry (6-8) to determine herbicidal content of polymeric microcapsules of alachlor and atrazine. HPTLC has been used for the quantitfication of atrazine and simazine in water (7) and reverse-phase HPTLC (RP-HPTLC) has been used as a simple and direct method of analysis of soils for atrazine and its metabolites (8).

MATERIALS AND METHODS

Chemicals and Reagents.

Technical atrazine [mp 175-177 °C; lit mp: 176 °C (9)] was provided by CIBA, Greensboro, North Carolina. Technical alachlor (provided by Monsanto, St. Louis, Missouri) was recrystallized from 95% ethanol affording material of mp 39.1-41.9 °C (lit mp: 39.5-41.5 °C) (9). Samples of the 88% hydrolyzed polyvinyl alcohols Airvol 205 (low viscosity) and Airvol 523 (medium viscosity) were provided by Air Products and Chemicals, Inc., Allentown, Pennsylvania. Stock 0.5% solutions of Airvol 205 and 523 were prepared by adding the polyvinyl alcohol to the vortex of stirred cold water in a steady stream followed by heating at 85 °C for about 30 minutes. The following polymers were purchased from Aldrich Chemical Company, Inc.: cellulose acetate butyrate, butyryl content 17%, Tm 235 °C (CAB); ethyl cellulose, ethoxyl content 48%, viscosity (5% solution in 80/20 toluene/ethanol) 22 centipoises [EC22]; ethyl cellulose, ethoxyl content 48%, viscosity 100 cps (EC100); poly(methyl methacrylate), low molecular weight (PMML); poly(methyl methacrylate), medium molecular weight

(PMMM). HPLC Reagent grade dichloromethane (DCM) and methanol (MeOH) were used as solvents.

Preparation of Polymeric Microcapsules.

Atrazine and alachlor were microencapsulated within cellulose acetate butyrate, ethyl cellulose of two different viscosities, and low and medium molecular weight poly(methyl methacrylate) by the solvent evaporation process using two different emulsifiers as previously reported (5).

In subsequent discussions, a polymeric microcapsule formulation will be referred to in abbreviated form, such as CAB-205, indicating the use of the polymer cellulose acetate butyrate and the emulsifier Airvol 205.

The herbicidal content of all the polymeric microcapsules prepared was determined by elemental analysis at the time of preparation. Based upon the amounts of materials used, each of the polymeric microcapsule formulations should contain 20% active ingredient. Determination of the herbicidal content of the CAB, EC22, and EC100-523 atrazine formulations was based upon nitrogen and chlorine microanalyses. The atrazine content of the PMML, PMMM, and EC100-205 formulations was determined from nitrogen microanalyses only. High values for the chlorine content of the PMML-523 and PMMM-523 formulations suggested the presence of residual dichloromethane. Chlorine microanalysis for PMML-205, PMMM-205, and EC100-205 were not done due to sample insolubility.

Preparation of Samples for Thin Layer Chromatography.

Standard solutions of alachlor (1.008 μg/μl) and atrazine (1.000 μg/μl) were prepared in methanol. Solutions of

microcapsule formulations were typically prepared by dissolving 250.0 mg of microcapsules in 100 ml of methanol or DCM giving an effective microcapsule concentration of 10.00 µg/4µl. All polymeric formulations dissolved readily in DCM, but only EC formulations dissolved completely in methanol. At least 24 hours were allowed for complete dissolution of alachlor or atrazine from the partially soluble CAB, PMML, and PMMM formulations. Blank solutions were prepared by dissolving 200.0 mg of each of the polymers in 100 ml of DCM. None of the polymers were detectable at the UV wavelengths used.

Thin Layer Chromatography.

TLC was performed on C-18 high performance reverse-phase Uniplates (10 X 20 cm, 150 micron thickness, scored, RP18F; Analtech Inc., Newark, Delaware). Standards and sample extracts or solutions were drawn into microcapillary pipets (1.0, 2.0, and 4.0 µl) and applied with a Nanomat III (Camag, Inc.). The mobile phase for alachlor experiments was MeOH:H_20 (85:15), and for atrazine experiments it was MeOH:H_20 (70:30). Each plate was spotted with 1.0, 2.0, 3.0 and 4.0 µl of the applicable standard solution and with 4.0 µl of each of seven sample solutions in duplicate. In a few instances, the seventh sample was spotted in triplicate. Spotted plates were equilibrated in a development tank containing the mobile phase for 20 min. prior to development. Plates were developed for a distance of 10 cm, dried for at least 15 min., and scanned at 200 nm for alachlor or 220 nm for atrazine with a variable wavelength Shimadzu CS9000U Dual-Wavelength Flying Spot Scanner. Development time was 25 min. for alachlor experiments and 30 min. for atrazine experiments.

Statistical Analysis.

The RP-HPTLC standard curves were analyzed by linear regression analysis (10). The mean alachlor or atrazine concentration in each sample was determined in micrograms and converted to a percentage (based on weight). Variance components were computed using the Maximum Likelihood method available in Proc Varcomp (10) to compare variability among HPTLC runs to variability between duplicate samplings of the same formulation. The t tests (10) were conducted separately on data collected for each formulation, using averages of duplicate samples as a run, to determine whether or not solvent significantly affected the result. Standard errors were calculated for determinations by HPTLC runs, using averages of duplicate samples as a run, and compared to the standard error of % N and % Cl determinations combined, using the homogeneity of variance test (10). The average of HPTLC runs collected for each formulation was paired with the % N (% Cl) result and a paired t-test (10) across all formulations was conducted to determine how closely HPTLC and % N (% Cl) results agreed.

RESULTS AND DISCUSSION

The results of the determination of alachlor content of polymeric microcapsules is given in Table I. The alachlor content in each formulation is given as a percentage (w/w). The percentage alachlor as determined by elemental analysis (based on % N and on % Cl) is given for comparison purposes. All samples listed under the same formulation heading were prepared from the same batch of polymeric microcapsules. Samples denoted by the A, C, and D prefixes were prepared from fresh microcapsules.

TABLE I

Determination of Herbicidal Content of Polymeric Alachlor Microcapsules by RP-HPTLC and Densitometry[1]

Formulation	Sample	Solvent	From RP-HPTLC Mean	SE	From Elemental Analysis Mean	Standard Error	p-value[2] Mean	Variance
EC100-205	A1, A2	DCM	17.52[3]	0.39	18.45[4]	2.85	0.5265	0.0173
	C1, D2	MeOH	18.11[3]	0.20	18.45[4]	2.85	------	-----
EC22-205	A3, A4	DCM	17.65[3]	0.32	20.75[4]	2.35	0.0273	0.0170
	C2, D1	MeOH	17.88[3]	0.28	20.75[4]	2.35	------	-----
CAB-205	A5, A6	DCM	18.88[3]	0.32	16.25[4]	6.15	0.3279	0.0000
	D3, D4	MeOH	20.12[3]	0.40	16.25[4]	6.15	------	-----
EC100-205	B1, B2	DCM	16.28[5]	0.24	20.20[4]	1.20	0.0016	0.0700
EC22-205	B3, B4	DCM	16.46[6]	0.37	19.70[4]	2.90	0.0593	0.0118
CAB-523	B5, B6	DCM	19.17[5]	0.31	21.35[4]	0.55	0.0130	0.7099
PMML-523	B7	DCM	19.83[7]	0.45	24.80[4]	0.30	0.0041	0.7206

[1]Alachlor content in each formulation is given as a percentage (w/w). [2]p-Value <0.05 indicates a significant difference between the RP-HPTLC method and elemental analyses. [3]Mean and standard error (SE) based on n = 8 runs. [4]Mean and SE based on n = 2 runs. [5]Mean and SE based on n = 6 runs. [6]Mean and SE based on n = 7 runs. [7]Mean and SE based on n = 3 runs.

Samples prepared from 4-year old microcapsules are denoted by the B prefix. Duplicate samplings (e. g., A1 and A2 and C1 and D2) were taken from the same batch of microcapsules to test for homogeneity. Samples prepared in dichloromethane (DCM) solution are designated by the prefixes A and B, and preparations in methanol (MeOH) solution are designated by C and D. There were a total of 13 separate HPTLC plate developments or runs. Samples denoted by A were spotted on the same plate, B samples were spotted on the same plate, and C and D samples were spotted together on the same plate. Each individual sample was spotted on at least three different plates. None of the developed TLC plates showed any impurities or decomposition products, indicating long-term stability of the microcapsules.

The following data were obtained from the 13 plate developments. The R-square for the alachlor standard curve varied between 0.990 and 0.999 with a mean of 0.996. The coefficient of variation (%CV) for the duplicate spottings was always under 10%, under 5% ninety-two percent of the time, and under 2% sixty-two percent of the time. These data indicate very high reproducibility in the spotting technique. There was no significant difference in %CV between methanol solutions and DCM solutions, indicating that the higher volatility of DCM was not a source of error in the spotting.

Statistical analysis of the data in Table I leads to the following conclusions. Variability among HPTLC runs is greater than variability between duplicate samplings of the same formulation, as indicated by variance component estimates (4.20 and 0.34 respectively). There is no statistical difference ($p>.19$) between the results obtained with the two solvents, DCM (A samples) and MeOH (C and D samples), as shown by the t-test conducted for

each formulation. The variability among percentage alachlor determinations by HPTLC runs is significantly less than that between the determinations based on % N and on % Cl for CAB-205 only; however, numerically the variability is smaller for the RP-HPTLC method in every case except for PMML-523. Additional elemental analyses would be required to make more thorough variability comparisons. It must be noted that of a total of ten different formulations, the four 4-year old alachlor formulations chosen for examination had the lowest variability between % N and % Cl determination of herbicidal content. Finally, the RP-HPTLC results are generally more in agreement with percentage determination based on % N (p=.9935) than on % Cl (p=.0001), as shown by paired t tests.

The results of the determination of atrazine content of 4.5- year old polymeric microcapsules is given in Table 2. Samples dissolved in methanol are denoted by the prefix E and those dissolved in DCM are denoted by the prefix F. The numerical suffixes denote the same polymeric formulation. The five formulations listed showed no evidence of impurities or decomposition products. Four unlisted formulations (EC22-205, EC22-523, EC100-205, and EC100-523) showed predominantly atrazine (Rf 0.414-0.44), but there was a significant amount of a second compound (Rf 0.501-0.53). The tenth formulation (PMMM-205) showed a compound with Rf 0.475, but no atrazine. Neither of these two unknowns is deethylatrazine, deisopropylatrazine, or hydroxyatrazine, as demonstrated by spotting of authentic samples of these three metabolites and comparison of Rf values (8).

The following data were obtained from the six plate developments employed. The R-square for the atrazine standard

TABLE II

Determination of Herbicidal Content of Polymeric Atrazine Microcapsules by RP-HPTLC and Densitometry[1]

Formulation	Sample	Solvent	From RP-HPTLC Mean	From RP-HPTLC SE	From Elemental Analysis Mean	From Elemental Analysis Standard Error	p-value[2] Mean	p-value[2] Variance
CAB-523	E1	MeOH	20.13[3]	0.32	20.40[4]	1.10	0.7901	0.2116
CAB-205	E2	MeOH	19.57[3]	0.19	20.00[4]	1.80	0.7714	0.0311
PMML-205	E6	MeOH	21.13[3]	0.63	20.1[5]	----	0.5003	-----
PMML-523	E7	MeOH	22.53[3]	0.50	19.7[5]	----	0.1045	-----
PMMM-523	E9	MeOH	21.07[3]	0.60	18.8[5]	----	0.0828	-----
CAB-523	F1	DCM	15.57[3]	0.62	20.40[4]	1.10	0.0242	0.5659
CAB-205	F2	DCM	15.07[3]	0.32	20.00[4]	1.80	0.0363	0.0311
PMML-205	F6	DCM	16.23[3]	0.51	20.1[5]	----	0.0227	-----
PMML-523	F7	DCM	17.33[3]	0.18	19.7[5]	----	0.0215	-----
PMMM-523	F9	DCM	15.70[3]	0.50	18.8[5]	----	0.1045	-----

[1]Atrazine content in each formulation is given as a percentage (w/w). [2]p-Value <0.05 indicates a significant difference between the RP-HPTLC method and elemental analyses. [3]Mean and standard error (SE) based on n = 4 runs. [4]Mean and SE based on n = 2 runs (nitrogen and chlorine microanalyses). [5]Nitrogen microanalysis only.

curve varied between 0.991 and 0.999 for 5 plates and was 0.971 for the sixth. The %CV for the duplicate spottings was always under 10%, under 5% eighty-five percent of the time, and under 2% forty-one percent of the time. These data indicate high reproducibility in the spotting technique.

In contrast to the results obtained for alachlor solutions, the percentage atrazine as determined with DCM solutions of the polymeric microcapsules was uniformly and markedly lower ($p<.0028$) than that determined with methanol solutions. However, the mean percentage of atrazine as determined with methanol solutions was in excellent agreement with the theoretical percentage of 20% and with the percentage derived from % N microanalysis.

CONCLUSIONS

A method employing reverse-phase high performance thin layer chromatography with densitometry in the determination of herbicidal content of alachlor and atrazine polymeric microcapsule formulations has been developed. The method is rapid, accurate, reproducible, and inexpensive (on a per sample basis) and has the added advantage in that impurities, metabolites, and decomposition products (which may yield falsely high percentages of herbicidal content when determined by elemental analyses) are readily detected. In the determination of the herbicidal content of alachlor formulations dissolution of samples in either methanol or dichloromethane gives comparable results. Methanol is the solvent of choice in the analysis of polymeric atrazine formulations. The method should be adaptable to the analysis of formulations of other UV-active herbicides, such as metribuzin and cyanazine.

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