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DEVELOPMENT OF A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR FLUROXYPYR HERBICIDE AND METABOLITES USING COMPUTER SIMULATION WITH DRYLAB G SOFTWARE

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SUMMARY

The herbicide fluroxypyr 1-methylheptyl ester was separated from its acid form (fluroxypyr) and two soil metabolites (a pyridinol and a methoxypyridine) by high-performance liquid chromatography (HPLC) with the aid of Drylab G. A difference in retention time of at least 6 min between each compound was achieved, to allow complete separation of radiolabeled components during collection of 1-min fractions. The total elution time was 31 min. Actual HPLC retention times differed from DryLab G predictions by 0.5 min or less.

INTRODUCTION

Fluroxypyr-MHE (1-methylheptyl ester) is the active ingredient of Starane® herbicide[a], which is used in Europe for the control of broadleaf weeds in small grains. After fluroxypyr-MHE has been sprayed onto soil, it hydrolyzes rapidly to fluroxypyr, which degrades microbially to carbon dioxide via two metabolites 4-amino-3,5-dichloro-6-fluoropyridin-2-ol and 4-amino-3,5-dichloro-6-fluoro-2-methoxypyridine (Fig. 1)[1].

In order to evaluate the fate of this herbicide in the environment, laboratory soil degradation studies were to be performed using ^{14}C-labeled fluroxypyr-MHE. After various periods of incubation, the ^{14}C-containing soils were to be extracted with

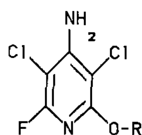

Fig. 1. Compounds used. R = $CH_2COOCH(CH_3)(CH_2)_5CH_3$ for fluroxypyr-MHE; R = CH_2COOH for fluroxypyr; R = CH_3 for the methoxypyridine metabolite; R = H for the pyridinol metabolite.

[a] Trademark of The Dow Chemical Company.

TABLE I

DETERMINATION OF DWELL VOLUME USING PREDICTIONS FROM 30- AND 60-MIN ELUTIONS AND COMPARISON OF PREDICTIONS WITH AN ACTUAL 90-MIN ELUTION

Component	*Retention time (min)*					
	Actual		*Predicted[a] for 90-min elution: dwell volume of*			*Actual for 90-min elution*
	30-min elution	*60-min elution*	*7.0 ml*	*7.5 ml*	*8.0 ml*	
Pyridinol	13.79	18.54	21.99	22.05	*22.11*	22.10
Fluoroxypyr	15.95	23.81	30.54	*30.63*	30.72	30.61
Methoxypyridine	19.47	29.69	*38.36*	38.44	38.53	38.32
Fluroxypyr-MHE	26.96	45.20	*62.06*	62.15	62.24	62.08

[a] Values in italics for predicted retention times (90-min gradient) indicate best fits with actual retention times for the 90-min gradient.

acidified acetone and subjected to high-performance liquid chromatographic (HPLC) separation and collection of 1-min fractions. An HPLC gradient was therefore needed that would not only separate the four compounds, but also maintain a 6–7-min interval between peaks. This latter requirement was needed to insure that levels of ^{14}C in at least two of the 1-min fractions following a peak would have decreased to background levels before the appearance of the next peak. If possible, a total elution time of about 30 min was desired. Linear gradients (30, 60 and 90 min; see Table I) were run, but none of these satisfied the needs of the present assay. Computer simulation[2,3] using the Drylab G software was therefore explored in an effort to achieve an improved gradient elution separation. This approach uses two initial experimental runs as computer input data, following which the computer can be used to explore the effects of various gradient conditions.

EXPERIMENTAL

HPLC system

Reversed-phase HPLC was performed on a Waters Assoc. Model 600E system controller and pump, with a Waters 10-μm μBondapak C_{18} column (10 cm × 0.8 cm I.D.) and a Waters Assoc. 484 variable-wavelength detector, set at 254 nm. The eluents were 1% acetic acid in water (solvent A) and 1% acetic acid in acetonitrile (solvent B). The flow-rate was 2 ml/min. Reference standards (obtained from Dow Chemical) were dissolved in acetonitrile to yield a solution containing 1 mg ml^{-1} each of fluroxypyr and fluroxypyr-MHE and 0.5 mg ml^{-1} each of the pyridinol and the methoxypyridine. Volumes of 50 μl were injected into the HPLC system with a Rheodyne 7125 syringe-loading sample injector.

Computer software

The DryLab G software (LC Resources, Lafayette, CA, U.S.A.) was used with an IBM-compatible personal computer.

TABLE II

HPLC GRADIENT PROGRAM PRODUCED USING DRYLAB G

Solvent B = 1% acetic acid in acetonitrile. All changes in solvent composition are linear.

Event	*Solvent B (%)*	*Time (min)*
At start of gradient	20.000	0.000
End of segment 1	20.001	5.000
End of segment 2	60.000	25.000
End of segment 3	100.000	26.000
End of last segment	100.001	36.000

Determination of dwell volume

As the dwell volume was not known for our HPLC system, it had to be determined empirically. A linear solvent gradient, running from 0 to 100% B in either 30, 60 or 90 min was used to generate three elution profiles of the four compounds. The retention times for the 30- and 60-min gradients were loaded into the DryLab program and the value for the dwell volume was adjusted by trial and error until the program was most closely able to predict the retention times for the 90-min gradient (Table I). For the pyridinol, a dwell volume of 8.0 ml best predicted the actual value obtained from the 90-min gradient, a 7.5-ml dwell volume best predicted the retention of fluroxypyr and 7.0 ml was appropriate for the methoxypyridine and fluroxypyr-MHE. An "average" value for the correct dwell volume was chosen as 7.5 ml.

Prediction and testing of the analytical gradient

Using a dwell volume of 7.5 ml, trial and error with the DryLab program was again used in constructing a two-step gradient for the desired elution of the four compounds (Table II). About 25 trial gradients were attempted during a period of several hours, compared with an estimated 1-week period had the different gradients been tested under actual HPLC conditions. Using an average dwell volume of 7.5 ml, a satisfactory separation of the four compounds was predicted (Table III). The predicted retention times matched closely the actual retention times observed by HPLC, thus providing a separation that was used successfully in the analysis of radiolabeled soil extracts. Fig. 2 (top) shows the simulated chromatogram for this separation

TABLE III

COMPARISON OF DRYLAB PREDICTIONS WITH ACTUAL HPLC RETENTION TIMES

Component	*Predicted retention time (min)*	*Actual retention time (min)*[a]
Pyridinol	8.52	9.05 (0.09)
Fluroxypyr	15.30	15.18 (0.06)
Methoxypyridine	21.08	21.13 (0.03)
Fluroxypyr-MHE	31.02	30.72 (0.00)

[a] Numbers in parentheses are standard deviations (n = 3).

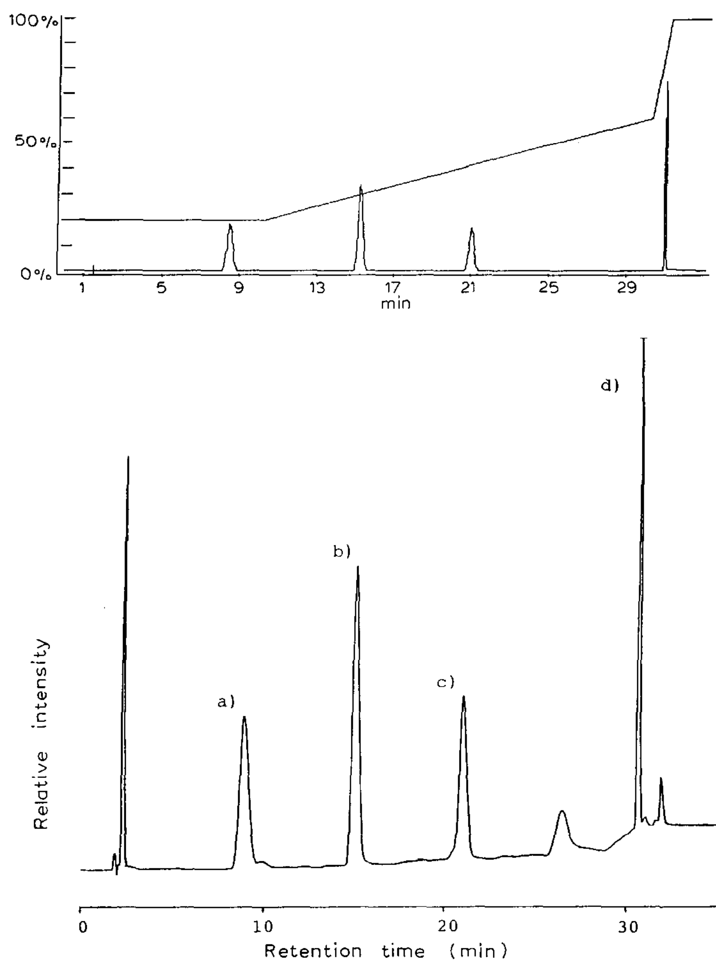

Fig. 2. Optimized separation of the sample (conditions as in table II). Top, predicted chromatogram (DryLab G); bottom, experimental chromatogram. Peaks: (a) pyridinol metabolite; (b) fluroxypyr; (c) methoxypyridine metabolite; (d) fluroxypyr-MHE.

provided by DryLab G, and Fig. 2 (bottom) is the experimental chromatogram for the same conditions.

An HPLC procedure for the assay of mixtures of flyroxypyr and its metabolites (with radiometric detection) has been developed. It was required that the various components be well resolved (6–7-min separation) in order to allow their complete resolution while maintaining a run time of about 30 min. This was achieved by means of a two-step gradient, designed through the use of computer simulation. The predicted, optimum separation agreed well with that found experimentally.

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