Determination of Oryzalin in Water-Sediment Systems

An analytical procedure for determining residual oryzalin at the 1-ppb level in water–sediment (runoff) samples is described in detail and thoroughly evaluated. Using extracting solutions with benzene–methanol ratios ranging from 3:1 to 9:1, we recovered 94–96% oryzalin from fortified water–sediment samples. After derivatization, no cleanup by column chromatography was required using the GC conditions described.

Oryzalin (3,5-dinitro-N^4,N^4-dipropylsulfanilamide) is a selective, preemergence, soil-surface-applied herbicide used to control most annual grasses and certain broadleaf weeds in soybeans, cotton, vineyards, ornamentals, and other crops (Berg, 1978). Compared with other dinitroaniline herbicides, oryzalin has a relatively high water solubility of about 2.5 ppm and a relatively low vapor pressure of about 1×10^{-7} mmHg at 30 °C (Probst et al., 1975). Gingerich and Zimdahl (1976) report the aerobic half-lives for soil-incorporated oryzalin to be 1.40 and 4.35 months at 30 and 15 °C, respectively. A review by Helling (1976) indicates that the dinitroanilines are strongly adsorbed on soil but that field losses of dinitroanilines, unless incorporated, can occur by sediment–water runoff. Because of these factors and the fact that oryzalin is often not incorporated after application, information is needed concerning oryzalin's pollution potential, i.e., its fate in agricultural runoff and other aquatic environments. Analytical methodology is, therefore, needed for oryzalin in water and runoff systems, because the previously reported method of Sieck et al. (1976) is suitable only for agricultural crops and soil. In this paper, we report an analytical procedure developed for determining oryzalin in runoff systems.

EXPERIMENTAL SECTION

Sample Preparation. Fortified sediment–water samples were prepared by adding 3 g of sediment (containing 1000 ng of analytical grade oryzalin) to 1 L of distilled water. Air-dried field soil, ground to pass a 0.5-mm sieve, was used to simulate sediment. The 1-L mixtures (prepared to contain 1 ppb oryzalin and 3000 ppm sediment) were stirred continuously for 15 min before and during all subsampling.

Subsampling and Extraction. Subsamples (100 mL) were transferred by pipet to 225-mL wide-mouth bottles. To these, we added 100 mL of extracting solvent (pesticide grade). Benzene only and six benzene–methanol solutions (9:1, 6:1, 3:1, 2:1, 1:1, and 0.5:1) were tested for their extraction efficiency. Extraction was accomplished by sonification (560-W peak output) for 1.5 min and partitioning in a separatory funnel. The water layer was drained, and the benzene layer was dripped slowly through anhydrous Na_2SO_4 into a 250-mL flask and then concentrated to almost dryness (<1 mL), using a stream of dry air. Methanol (25 ml) was added before derivatization.

Derivatization. Oryzalin in the methanol solution was converted to its dimethyl derivative, using anhydrous Na_2SO_4 and CH_3I, as described by Sieck et al. (1976). After the derivatization reaction was complete (overnight), we added 50 mL of distilled water and the mixture was transferred to a 125-mL separatory funnel. The flask was rinsed with three 15-mL aliquots of benzene, and the rinses were added to the separatory funnel. The separatory funnel was shaken vigorously and allowed to stand for 30 min. As before, the water layer was drained, and the benzene layer was dripped slowly through anhydrous Na_2SO_4. The separatory funnel was rinsed with 25 mL of benzene, which was also dripped through the Na_2SO_4. The benzene solution was concentrated by dry air to 10 mL for gas chromatographic analysis. Cleanup by alumina column chromatography (Sieck et al., 1976) was not needed with the runoff samples and GC column and conditions used in this study. This does not preclude the need for cleanup by column chromatography in all instances. The efficiency of the derivatization reaction was determined using four control samples, each containing 100 ng of oryzalin, with each set of fortified sediment–water samples.

Table I. Percent Recovery of Oryzalin (1-ppb Level) from Sediment-Water Samples[a]

benzene-methanol ratio (v/v)	oryzalin recovery, %
0.5:1	52.3d ± 1.9
1:1	67.3c ± 1.0
2:1	87.2b ± 1.2
3:1	96.1a ± 0.8
6:1	94.0a ± 0.7
9:1	96.1a ± 1.0
benzene only	85.8b ± 1.2

[a] Each value is the mean of eight replicates. Values followed by a common letter are not significantly different at the 5% probability level, according to the Duncan multiple-range test.

GC Analysis. Dimethyloryzalin in the benzene solution was analyzed on a 180-cm × 6-mm o.d. × 4-mm i.d. U-shaped column packed with 5% OV-1 on 100/120 mesh Chromosorb W (HP, AW, DMCS). Carrier gas was prepurified nitrogen at 180 mL/min. Column oven, inlet, and ^{63}Ni electron-capture detector temperatures were 220, 230, and 275 °C, respectively. Under these conditions, the retention time for dimethyloryzalin was 5.1 min. All recoveries were expressed as percent oryzalin recovered and were corrected for the difference in molecular weight of oryzalin and dimethyloryzalin, as well as for derivatization efficiency.

RESULTS AND DISCUSSION

The derivatization procedure was determined to be 86.5 ± 5.0% efficient based on 28 control samples. This agreed closely with that (88%) reported recently by Sieck et al. (1976). The percent recovery of oryzalin from 1 ppb fortified sediment–water samples using various benzene–methanol extracting solutions is shown in Table I. These data showed that oryzalin recoveries were highest for benzene–methanol ratios in the range of 3:1 to 9:1. Recoveries decreased sharply for ratios of less than 3:1. With benzene only as the extracting solution, oryzalin recovery was only slightly lower (about 86%). These results indicated that some methanol is needed to achieve quantitative recovery (>90%); however, too much methanol reduces extraction efficiency.

Attempts to shorten and further simplify the overall extraction–derivatization procedure by derivatizing oryzalin in benzene, instead of methanol, were totally un-

successful. Benzene probably lacks sufficient solvent polarity.

Recommendations. Field samples of runoff should be at room temperature and continuously stirred during subsampling. Oryzalin should be extracted with 3:1 to 9:1 benzene–methanol solution; however, based on present pesticide-grade solvent costs, 3:1 benzene–methanol is more economical than 9:1. The GC column should have at least the resolution of the one described in the Experimental Section.

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Separation of Soybean Lipoxygenase and Peroxidase by Hydrophobic Chromatography

Separation of lipoxygenase from peroxidase in legumes has posed some difficulties in using conventional ion-exchange chromatography. With hydrophobic chromatography, crude extracts of soybean flakes showed selective, reversible binding of the two enzymes onto Phenyl Sepharose CL-4B resins. Elution of the enzymes from the resin was performed by a stepwise decrease in the concentration of the equilibration buffer at pH 6.8. Lipoxygenase eluted from the hydrophobic column before peroxidase, resulting in clean separation, high yield, and substantial purification of both enzymes. Disc gel electrophoresis showed that lipoxygenase was considerably purer than peroxidase, and the two enzymes were clearly separated from one another without any cross contamination. An acid-shocking phenomenon was observed in the detection of the peroxidase isozyme forms.

Hydrophobic chromatography is a technique which allows the separation of proteins by making use of the differences in size and distribution of available hydrophobic pockets or regions on the proteins (Shaltiel, 1974). This technique has been used in studies characterizing food enzymes such as peach polyphenol oxidase (Flurkey and Jen, 1978). The resolution potential of hydrophobic chromatography is not exhausted by choosing the most appropriate spacer arms; further resolution can be achieved by specific elution conditions.

Lipoxygenase (linoleate: O_2 oxidoreductase, EC 1.13.1.13.), isolated by Theorell et al. (1946), catalyzes the oxidation of various *cis,cis*-1,4-pentadiene systems into hydroperoxides by means of molecular oxygen (Holman and Bergström, 1951). Wolf (1975) and Rackis et al. (1972) have shown that the enzyme is closely related to flavor production and the off-flavor of soybean protein products.

Traditionally, soybean lipoxygenase was purified by conventional techniques such as ammonium sulfate fractionation, molecular filtration, and ion-exchange chromatography (Christopher et al., 1970; Johns et al., 1973). These techniques are reliable but the processes are usually slow and the yields are relatively low. Catsimpoolas (1969) achieved a 230-fold purification of soybean lipoxygenase using the isoelectric focusing technique. Grossman et al. (1972) applied affinity chromatography through a linoleyl aminoethyl agarose column and obtained near homogeneous soybean lipoxygenase. On the other hand, Allen et al. (1977) used a number of aminohexyl agarose derivatives of unsaturated fatty acids for a one-stage purification of soybean lipoxygenase-1 and obtained a purification factor of 16. They suggested that in their affinity chromatographs, the major specific substrate-binding force is not a hydrophobic interaction with the saturated hydrocarbon moiety of the substrate. Wheeler and Wallace (1978), using wheat germ lipoxygenase, have shown that the enzyme could be bound to Teflon or epoxy-coated surfaces through hydrophobic interactions.

In this report, we showed that soybean lipoxygenase can be bound to a hydrophobic resin. Further, we showed that in spite of the fact that both soybean peroxidase and lipoxygenase are retained on a hydrophobic column, they can be made to detach selectively from the column by changing the nature of the eluting solvent, thus resulting in clean separation and substantial purification of both enzymes.

MATERIALS AND METHODS

Materials. Defatted soybean flakes were obtained at a local market. Linoleic acid (99%) was purchased from Sigma Chemical Company. Phenyl Sepharose CL-4B was obtained from Pharmacia Fine Chemicals. All other materials used in this study were commercial products of reagent grade quality.

Enzyme Extraction. Lipoxygenase extraction was a modification of the method described by Christopher et al. (1970). Defatted soybean flakes (30 g) were homogenized with 300 mL of sodium phosphate buffer 0.05 M, pH 6.8) in a prechilled Virtis homogenizer. The soybean homogenate was stirred for 90 min at room temperature.

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