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Determination of diflubenzuron residues in water by solid-phase extraction and quantitative high-performance thin-layer chromatography

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ABSTRACT

A high-performance thin-layer chromatography (HPTLC) method using channeled, preadsorbent silica gel plates and Bratton–Marshall detection reagent was combined with C_{18} solid-phase extraction for quantification of diflubenzuron residues in water. The sensitivity of the technique for diflubenzuron was 0.1 μg, and residues in water at a concentration of 50 μg/l were determined with recoveries of 95–97% and relative standard deviations of 2–3%. Residues could be semi-quantitatively determined at concentrations down to 125 ng/l.

INTRODUCTION

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] (DFB) is a substituted benzoylurea insecticide that acts by interference with deposition of insect chitin. The only published thin-layer chromatography (TLC) method for DFB [1] qualitatively determines residues in water by methylene chloride extraction, separation on homemade silver-impregnated alumina layers, and detection by irradiation with UV light.

In an earlier paper [2], we reported the densitometric quantification of seven substituted urea herbicides, which have structures related to DFB, on C_{18} reversed-phase thin layers using Bratton–Marshall detection reagent after *in situ* hydrolysis to produce aromatic amines. This paper describes the determination of DFB residues in water by a similar densitometric TLC method after isolation on a C_{18} solid-phase extraction (SPE) column using procedures analogous to those we reported previously for the SPE of organochlorine insecticides [3], organophosphorus insecticides [4], and chlorinated herbicides [5].

EXPERIMENTAL

Pesticide solutions

Diflubenzuron standard was obtained from the EPA Pesticide Repository (Research Triangle Park, NC, USA). A stock standard solution was prepared in ethyl acetate at a concentration of 1.0 mg/ml, and this solution was quantitatively diluted with ethyl acetate to prepare a 0.10 mg/ml TLC standard solution and a 0.050 mg/ml spiking solution.

TLC procedure

TLC was carried out on 10 × 20 cm Whatman (Clifton, NJ, USA) LHP-KDF high-performance silica gel plates containing 19 channels and a preadsorbent spotting area. Plates were precleaned by development with methylene chloride–methanol (1:1). Standard and sample solutions were applied using a 25-μl Drummond (Broomall, PA, USA) digital microdispenser. Plates were developed for a distance of 6 cm beyond the preadsorbent-silica gel interface (*ca.* 12 min.) with ethyl acetate–toluene

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(1:3) in a paper-lined, solvent-equilibrated glass HPTLC chamber, and the plate was removed from the chamber and air-dried. Zones were detected as described earlier [2] by spraying in turn with 6 *M* ethanolic hydrochloric acid, 1% sodium nitrite in ethanolic HCl, and 1% ethanolic *N*-(1-naphtyl)ethylenediamine dihydrochloride. The layer was covered with a clean glass plate and heated at 180°C for 10 min after the first spray. The detection procedure is most successful when the spray solutions are prepared freshly within 4 h of use. DFB zones were scanned at 550 nm using a Shimadzu CS-930 densitometer in the single-beam, single-wavelength reflectance mode.

Water analysis

Recovery samples were prepared at a concentration of 50 μg/l by adding 1.0 ml of the spiking solution to exactly 1 l of water known form previous analysis to contain no DFB. The SPE method was adapted from an unpublished procedure supplied by Solvay Duphar B.V. (Weesp, Netherlands) [6]. A C_{18} disposable SPE column (J. T. Baker, Philipsburg, NJ, USA, No. 7020-3, 3 ml) was connected to a 75-ml reservoir, placed in a Baker-10 vacuum manifold operated at 15 inches of Hg, and washed in turn with 5-ml portions of acetonitrile, methanol, and deionized water. The 1 l water sample was passed through the column, followed by 35 ml of acetonitrile–water (3:7). The column was taken from the manifold and the reservoir removed, and the DFB was eluted with 2 ml of acetonitrile into a 2-ml graduated vial with a tapered bottom using gentle pressure from a rubber bulb or syringe. The vial was clamped in a 40°C water bath and the solution evaporated just to dryness under a stream of nitrogen gas. The residue was dissolved in exactly 1.0 ml of ethyl acetate to prepare the sample solution for TLC analysis.

Duplicate 5.0-μl portions from the 1 ml reconstituted sample solution were spotted on a TLC plate along with 1.0, 2.0, 4.0, 8.0 , and 12.0 μl (100–1200 ng) of the TLC standard. After development, detection, and scanning, the equation of the calibration curve (peak area of standards *vs.* weight spotted) was calculated, and the weight of DFB in the sample zones was interpolated from the standard curve. The percent recovery from spiked samples was calculated by dividing the average weight of DFB in the duplicate sample aliquots by the theoretical weight for 100% recovery (50 μg · 5 μl/1000 μl = 250 ng) and mulitplying by 100.

RESULTS AND DISCUSSION

The HP silica gel layer was found to be superior to the C_{18} layer used earlier [2] for the determination of substituted urea herbicides in terms of spot definition and detection sensitivity. On silica gel, DFB was detected as a compact purple-blue band on a white background with an R_F value of 0.40. The three detection solutions should not be sprayed too heavily or the spots will be blurred and the layer may pucker; spots appear as soon as the third solution, *N*-(1-naphthyl)ethylenediamine, is sprayed and reach maximum intensity within about 15 min.

The *in situ* spectrum of a sprayed 1.2-μg standard spot was obtained using the spectral mode of the densitometer, and the wavelength of maximum absorption was found to be 550 nm. In all subsequent analyses, DFB zones were scanned at this wavelength as soon as detection spray 3 dried, because the plate background becomes irreversibly purple after about 30 minutes.

The calibration equation calculated from the areas of the five standards typically had linearity coefficient (R) values of 0.97–0.99. Since slope and intercept values are somewhat variable, bracketing standards were applied and a separate calibration equation was calculated for each plate used to analyze samples.

Carbopack graphitized carbon cartridges were shown [7] to be more efficient than C_{18} for the extraction of phenylurea herbicides from water. However, the C_{18} SPE procedure proved to efficiently extract the less polar DFB from water and provided a quick and convenient alternative to the usual separatory funnel extraction prior to TLC. In the SPE method, the 30% aqueous acetonitrile eluent removes co-extracted impurities more polar than DFB, while the DFB is retained on the column. DFB is then completely eluted with 2 ml of acetonitrile, thereby achieving a 500-fold concentration increase from a 1-l water sample.

Recovery studies were carried out using 1-l water samples fortified with 50 μg of DFB (50 μg/l). A 5-μl aliquot from the 1000-μl reconstituted sample was spotted for TLC analysis, which represented

250 ng if recovery was 100%. Assuming that DFB quantities as low as 0.10 μg can be detected on the silica gel plate and a recovery of 90% through the SPE column, the ultimate sensitivity of the method for 1 l of water if the entire reconstituted extract residue was spotted would be approximately 111 ng/l. However, because of the experimental difficulties involved in dissolving and spotting the entire residue and working at the lowest sensitivity level of the detection method, results at this concentration would be semi-quantitative at best.

Three duplicate samples each of deionized water and local river water spiked at 50 μg/l were analyzed to test the accuracy and precision of the method. The average recovery (± S.D.) was 95 ± 2% for the deionized water and 97 ± 3% for the river water. The percentage difference between the areas of the duplicate sample aliquots spotted ranged from 2.8–8.1% with a mean of 5.9%. One sample of river water was spiked at 125 ng/l, and recovery was estimated to be 83% when the analysis was carried out after reconstituting the residue in 50 μl of ethyl acetate and spotting the entire residue onto the preadsorbent.

The earlier TLC method [1] claimed a detection limit of 0.1 μg of DFB on an absolute basis and a concentration detection limit of 2 μg/l. To achieve this limit, 100% recovery and a 50-μl sample would be required, but neither the sample size nor recovery were specified in the paper [1]. The method involved homemade silver-impregnated alumina layers, which are difficult to prepare reproducibly and turn black quickly on storage, and separatory funnel extraction. Since the layer did not contain a preadsorbent spotting area, precise application of a 50-μl sample in a narrow initial zone would be difficult and time consuming. The SPE–HPTLC method is much faster and convenient since it involves disposable C_{18} extraction columns and application of only 5 μl of sample solution to a commercial preadsorbent plate, and recovery studies show it is a reasonably accurate and precise quantitative procedure.

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