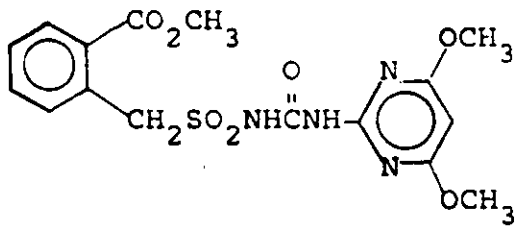
INTRODUCTION

An analytical method has been developed for determining residues of rice herbicide candidate DPX-F5384 in soils. This compound, structure below, is the active ingredient of Du Pont's "Londax" rice herbicide.



DPX-F5384

Methyl 2-[(4,6-dimethoxy pyrimidin-2-yl)aminocarbonyl] aminosulfonyl methyl benzoate

The method is based on the liquid chromatographic measurement of DPX-F5384, using a variable wavelength ultraviolet spectrophotometer detector. The compound is initially extracted from the soil sample with pH 10 water (deionized or distilled water is adjusted to pH 10 with ammonium hydroxide) followed by organic solvent partitioning and cleanup and by collecting the DPX-F5384 on a disposable C_{18} Bond Elut™. After elution, the DPX-F5384 residue is determined by High Performance Liquid Chromatography (HPLC). The method is sensitive to 1 ppb based on a 25 gram sample. Satisfactory recoveries have been demonstrated over the 1 ppb to 5 ppb range.

APPARATUS AND REAGENTS

A Waring commercial blender (Fisher Scientific Co., Pittsburgh, PA, Ca. No. 14-509-19) with a glass container (1-liter) was used to mill the dried soils and then to homogenize the samples during the pH 10 water extraction.

An International centrifuge model BE-50 (International Equipment Co., Boston, MA) with 200-mL capacity thick-wall glass centrifuge bottles was used to centrifuge the samples.

The pH of the samples was adjusted using a Fisher Accument® pH Meter, Model 810 (Fisher Scientific Co., Pittsburgh, PA., Ca. No. 13-636-810).

Rotary evaporators with temperature-controlled water baths equivalent to Rotavapor R® (Fisher Scientific Co., Pittsburgh, PA, Cat. No. 9-548-151) were used to evaporate and concentrate solutions. Round-bottom evaporating flasks were used with the rotary evaporators.

Acetonitrile, n-hexane, isopropyl alcohol, methanol, methylene chloride, and toluene were "HPLC" grade manufactured by Fisher Scientific Co., Pittsburgh, PA.

Glacial acetic acid, hydrochloric acid and ammonium hydroxide were "Reagent ACS" grade manufactured by Fisher Scientific Co., Pittsburgh, PA.

Water should be deionized or distilled. Chlorinated tap

water should not be used because residual chlorine may cause decomposition of DPX-F5384*.

Disposable C_{18} Bond Elut® extraction columns (Analytichem International, Harbor City, CA Part No. 607303) were used to collect and concentrate DPX-F5384 from aqueous solutions.

Solvent extractions were performed in 500 mL separatory funnels with Teflon® plugs (Fisher Scientific Co., Pittsburgh, PA, Cat. No. 10-437-10D).

Millex®-HV 0.45 µm Filter Unit, single use, non-sterile, solvent resistant, Durapore® Membrane (Millipore Corporation, Bedford, MA, Cat. No. SLHV025NS) were used to filter all samples prior to injection into the HPLC.

DPX-F5384 analyses were performed on Du Pont Instruments, Series 8800 Gradient Controller, Chromatographic Pump Model 861, Column Oven Model 851, and a U.V. Spectrophotometer Model 852 (Du Pont Instruments Division, Wilmington, DE). The column was a 4.6-mm x 25-cm Du Pont Zorbax® SIL. The recorder was a Perkin Elmer R100 (Norwalk, CT).

The reference standard of DPX-F5384 was obtained from the Agriculture Products Department, E. I. Du Pont de Nemours & Co., Inc., Wilmington, DE.

A standard stock solution (100 µg/mL) of DPX-F5384 was

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* Letter R. V. Slates to T. E. Catka, November 7, 1984.

prepared by weighing 0.010 grams of the DPX-F5384 reference standard into a 100 mL volumetric flask and adjusting the volume with acetonitrile to the 100 mL mark.

The working standard of 1 µg/mL DPX-F5384 was prepared daily for fortification purposes by pipeting 1 mL of the 100 µg/mL stock solution into a 100 mL volumetric flask and adjusting the volume to the 100 mL mark with acetonitrile. Standards for HPLC analysis were prepared at 0.05, 0.25, and 0.50 µg/mL by diluting a 1.0-µg/mL standard solution of DPX-F5384 in HPLC mobile phase. The 1.0-µg/mL standard solution of DPX-F5384 in HPLC mobile phase was prepared daily by pipeting 1 mL of the 100-µg/mL stock standard into a 100-mL volumetric flask, evaporating the acetonitrile with a gentle stream of dry nitrogen or air, and adjusting to volume with HPLC mobile phase. This standard was further diluted to 0.025 µg/mL to permit fortification at 1 ppb.

The HPLC mobile phase consisted of:

720 mL n-hexane
110 mL isopropyl alcohol
110 mL methyl alcohol
55 mL acetonitrile
2 mL acetic acid
1 mL water

The HPLC column cleaning solutions consisted of 400 mL isopropyl alcohol, 400 mL methyl alcohol, 200 mL acetic acid, and 40 mL water.

EXPERIMENTAL PROCEDURE

Sample Preparation

The frozen soil samples are permitted to thaw and are placed in disposable aluminum trays to air dry at room temperature. Five to seven days are required for complete drying. The dried samples are milled individually in a waring blender, in small portions, then charged to a 1-gal plastic wide mouth bottle and rolled on a mechanical drum roller for 30 minutes, to permit representative sampling of each soil sample.

Extraction

Weigh a 25-g representative soil sample and transfer it to a 1-liter waring blender container. If the sample is to be a fortified control to determine recovery efficiency, pipet the required volume of a .025 µg/mL standard solution of DPX-F5384 in acetonitrile onto the soil sample. Evaporate the acetonitrile

with a gentle stream of dry air or nitrogen. Extract the sample with 325 mL of pH 10 water (deionized or distilled water is adjusted to pH 10 with ammonium hydroxide using a pH meter). Blend during extraction at medium speed for five minutes. Readjust the pH of the extract to 10.0 with ammonium hydroxide and blend for another five minutes. Transfer the blended mixture to glass centrifuge bottles (two), making certain the bottles in the centrifuge cups are balanced. Centrifuge at high speed (2700 rpm) for a full 5 minutes. Do not brake the centrifuge, rather let it slow gradually. Decant the supernatant through glass wool into a 600-mL beaker. Wash the glass wool with approximately 10 mL of pH 10 deionized water. The solution has a muddy appearance at this stage. Adjust the pH to 2.5 using 10% hydrochloric acid and a pH meter. Return the pH-adjusted solution to clean centrifuge bottles (balance the bottles) and centrifuge at high speed for 10 minutes. Again, permit the centrifuge to stop without braking. Decant the clear supernatant to a 500-mL separatory funnel. Extract the supernatant with 100 mL of toluene plus 0.5 mL of glacial acetic acid. By hand, give a vigorous 3 minute wrist action shake. Let the phases separate, then drain off the lower water phase into a beaker. If an emulsion occurs, it must be broken prior to attempting to separate the layers. This can be accomplished by centrifuging the samples at full speed (2700 rpm) for a few minutes. Decant the toluene phase in to a 500-mL round

bottom flask. Repeat the 100-mL toluene - glacial acetic acid extraction two more times. Combine all three toluene extracts and evaporate them to dryness on a rotary evaporator at 55 - 60°C under water aspirator vacuum.

Solvent Partitioning Cleanup

Redissolve the sample residue in 30 mL of pH 10-adjusted deionized water, sonicate, and pour the solution in to a 500-mL separatory funnel. Wash the round bottom flask again with 30 mL of pH 10 water, sonicate, and transfer the wash solution to the same 500-mL separatory funnel. Wash the water phase with 50 mL of a mixture of toluene/methylene chloride, 90/10, V/V, with a 2-minute vigorous hand shake. Let the phases separate, then drain off the lower water phase into a beaker. Discard the organic phase. Return the water phase to the separatory funnel and repeat the wash a second time. Drain off the lower water layer into the beaker and discard the organic phase. Adjust the water phase to pH 2.5 with 10% hydrochloric acid using a pH meter, and return the solution to the separatory funnel. Extract the DPX-F5384 from the water layer with 100 mL of a mixture of toluene/methylene chloride, 90/10, V/V, plus 0.5 mL glacial acetic acid. By hand, give a vigorous 3-minute wrist action shake. Let the phases separate, then drain off the lower water phase into a beaker. Transfer the organic phase to a 250-mL round bottom flask. Return

the water phase to the separatory funnel and repeat the extraction a second time. After phase separation, drain off and discard the lower water phase. Combine the two organic phases and evaporate them to dryness on a rotary evaporator at 55 - 60°C. The use of a water aspirator permits a rapid evaporation. The dried residue may be stored in a round bottom flask at 4°C for up to 10 days before the Bond Elut® cleanup phase.

Bond Elut® Cleanup and Concentration

Condition a disposable C_{18} (3 cm^3) Bond Elut® column by flushing it with 20 mL of acetonitrile, followed with 20 mL of deionized water. Wash the 500-mL round bottom flask containing the sample residue with 5 mL of pH 10 deionized water, and sonicate. With a disposable pipet, transfer the 5-mL sample to the column. Repeat with 2 more 5-mL pH 10 water washes. Sonciate each wash and transfer the washes to the Bond Elut® column. Pass the solution through the column. Liquid should flow through the Bond Elut® column slowly so that the effluent forms distinct drops, not a steady stream. Flow rate can be controlled by applying vacuum to the column. Wash the round bottom flask with 20 mL of deionized water, sonicate, and pass the 20-mL wash through the column. Pull air through the column for several minutes to remove residual water. Elute the DPX-F5384 from the

Bond Elut® column with 10 mL of acetonitrile, collecting the eluate in a 15-mL centrifuge tube. Evaporate to dryness with a stream of nitrogen. Set aside for later analysis.

HPLC Analysis

DPX-F5384 is determined by high performance liquid chromatography using a variable wavelength ultraviolet spectrophotometer detector by comparing the chromatographic peak height for DPX-F5384 in the sample solution with the corresponding peak heights for standard solutions containing known quantities of DPX-F5384. Alternatively, if high background is observed in the control sample, a photo conductivity detector, Tracor® Model 965, Tracor® Instruments, Austin, TX, may be used.

Condition the column by pumping the HPLC cleaning solution through the column and detector at 0.5 mL/min. for at least four hours. Then pump the HPLC mobile phase through the column and detector for about two hours to establish equilibrium between the column and the mobile phase.

Dissolve the dried sample in the 15-mL tube in 2 mL of HPLC mobile phase. Analyze samples and standards alternately by HPLC using the following conditions:

Column:	Du Pont Zorbax® SIL, 4.6 mm x 25 cm
Column Oven Temperature:	40°C
Mobile Phase:	Composition given in "Apparatus and Reagent" section
Mobile Phase Flow-Rate:	0.5 mL/min.
Injection Volume:	50 µL loop
Detector Wavelength:	237 nm
Chart Speed:	5 mm/min
Retention Time:	~12.4 minutes

Calculations

Standard curves of DPX-F5384 peak height versus µg DPX-F5384 injected are linear with zero intercept for injections of up to 0.50 µg/mL.

The peak height of the DPX-F5384 is measured in mm and the concentration of DPX-F5384 present in the sample is determined in µg/mL from a calibration curve similar to that of Figure 1.

$$\text{ppb DPX-F5384} = \frac{[\mu g/mL \text{ in sample sol.}] \times 2 \text{ mL} \times 1000 \text{ ng}/\mu g}{[\text{Sample weight in g}]}$$