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DU PONT LONDAX® HERBICIDE

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Determination of Rice Herbicide Candidate
DPX-F5384 in Rice Grain

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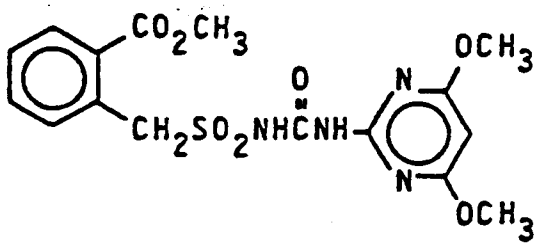
INTRODUCTION

This report documents the analytical method for determination of DPX-F5384 in rice grain. DPX-F5384 is a herbicide candidate for control of weeds in both lowland and dryland rice. DPX-F5384 has the following structure:



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

DPX-F5384

DPX-F5384 is isolated from polished or unpolished rice grain by methylene chloride extraction. The compound is separated from major interfering components by acetonitrile-hexane partitioning and by collection of the DPX-F5384 on a disposable C_{18} Bond Elut™ column from aqueous solution. After elution of the sample residue from the Bond Elut™ column by

acetonitrile, the residue for polished rice samples is transferred to mobile phase, and DPX-F5384 is determined by high performance liquid chromatography (HPLC).

Additional cleanup is required for unpolished rice samples. The residue eluted from the Bond Elut™ column is redissolved in water at pH 10 and is washed with toluene. After acidification of the aqueous phase to pH 4, DPX-F5384 is extracted into toluene, transferred to mobile phase, and is determined by HPLC.

The detection limit for DPX-F5384 in rice grain is 0.02 ppm. Average recovery efficiency for rice control samples fortified with DPX-F5384 at 0.02 to 0.08 ppm is 96%.

APPARATUS AND REAGENTS

A Tekmar Tissumizer® (Tekmar Co., Cincinnati, OH) with shaft number SDT 182 EN was used to homogenize samples during solvent extraction.

Millipore® 47-mm Teflon®-faced glass filters (Millipore Corp., Bedford, MA, Cat. No. XX 1004720) with 47-mm Millipore® Microfiber glass disk prefilters (Millipore Corp., Cat. No. AP4004705) were used with Fisher Filtrators® (Fisher Scientific Co., Pittsburgh, PA, Cat. No. 9-788) for all filtrations.

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.

Solvent extractions were performed with 125-mL separatory funnels with Teflon® plugs (Fisher Scientific Co., Pittsburgh, PA., Cat. No. 10-437-10B).

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

DPX-F5384 analyses were performed on a Du Pont Model 850 HPLC instrument fitted with a 4.6-mm x 25-cm Du Pont Zorbax® SIL column (Du Pont Instruments Division, Wilmington, DE), a Tracor Model 965 photoconductivity detector (Tracor Instruments, Inc., Austin, TX) with a mercury lamp, and a Hewlett Packard Model 3380A

integrating recorder (Hewlett Packard, Avondale, PA). To permit accurate balancing of the mobile phase flows through the reference and analytical cells of the detector, a metering valve (Nupro Co., Willoughby, OH, No. SS-2SA-TFE) was installed on the reference cell discharge line. The ion exchange resin tube and the micropump of the detector were not used because deionization of the mobile phase is not necessary and because the resin could actually introduce interfering contaminants into the mobile phase.

Disposable C_{18} Bond Elut™ extraction columns (Analytichem International, Harbor City, CA) were used to collect and concentrate DPX-F5384 from aqueous solution.

Acetonitrile, n-hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and toluene were "HPLC" grade manufactured by J. T. Baker Chemical Co., Phillipsburg, NJ.

Acetic 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.

The DPX-F5384 reference standard was synthesized and assayed in the Du Pont Agricultural Chemicals Department Research Division Laboratories.

The working standard of 1 µg/mL DPX-F5384 in methylene chloride was prepared daily for fortification purposes by diluting a 100-µg/mL stock solution. Standards for HPLC analysis were

prepared at 0.1, 0.2, and 0.4 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 methylene chloride with a gentle stream of dry nitrogen or air, and making to volume with HPLC mobile phase.

The HPLC mobile phase consisted of

750 mL	n-Hexane
125 mL	Isopropyl alcohol
100 mL	Methyl alcohol
25 mL	Acetonitrile.
2 mL	Acetic acid
1 mL	Water

The HPLC column cleaning solution consisted of 400 mL isopropyl alcohol, 100 mL glacial acetic acid, and 10 mL water.

EXPERIMENTAL PROCEDURE

Sampling and Extraction

To determine DPX-F5384 residues in polished or unpolished rice grain, weigh a 25-g representative sample and transfer it to a centrifuge bottle. If the sample is to be a fortified control to determine recovery efficiency, pipet the required volume of a 1.0-µg/mL standard solution of DPX-F5384 in methylene chloride onto the rice. Evaporate the methylene chloride with a gentle stream of dry air or nitrogen.

Extract the sample three times with 100 mL of methylene chloride per extraction using the Tekmar Tissumizer® to grind the sample to a fine powder. Typical grinding times for the three extractions are 60, 45, and 30 seconds respectively. After homogenizing the sample with the Tekmar Tissumizer®, centrifuge the sample, and filter it through a sintered glass filter with prefilter. Combine the three extracts for the sample and evaporate to dryness in a rotary evaporator at 35°C.

Solvent Partitioning Cleanup

Redissolve the sample residue in 50 mL acetonitrile and transfer the solution to a separatory funnel. Wash the acetonitrile phase three times with 50 mL n-hexane per wash. Shake the separatory funnel vigorously for at least two minutes for each wash and let phases separate well before discarding the hexane phase. Evaporate the acetonitrile solution to dryness on a rotary evaporator at 35°C.

Bond Elut™ Cleanup and Concentration

Condition a disposable C_{18} Bond Elut™ column by flushing it with 25 mL acetonitrile then with 25 mL of 0.15 M aqueous ammonium hydroxide solution. Dissolve the sample residue in 50 mL of 0.15 M ammonium hydroxide, and pass this solution through the Bond Elut™ column. Liquid should flow through the Bond Elut™ column slowly enough that the effluent forms distinct drops, not a steady stream. Flow rate can be controlled

by applying vacuum to the column. After passing the sample solution through the Bond Elut™ column, rinse the glassware which previously held the sample with 10 mL of 0.15 M ammonium hydroxide and pass this through the Bond Elut™ column too. Then pull air through the column to remove residual water. Elute the DPX-F5384 from the Bond Elut™ column with 10 mL of acetonitrile. Collect the eluate and evaporate to dryness. For polished rice samples, proceed to "HPLC Analysis" section.

Additional Cleanup for Unpolished Rice Samples

For unpolished rice samples, redissolve the sample residue in 50 mL of 0.15 M ammonium hydroxide solution. Wash the sample solution once with 50 mL of toluene. Shake vigorously for two minutes, centrifuge, and discard the toluene wash. Acidify the aqueous phase to pH 4.0 by slowly adding dilute HCl while monitoring pH with a pre-standardized pH meter. Extract the aqueous phase two times with 50-mL quantities of toluene. Shake vigorously for two minutes per extraction, centrifuge, and combine the toluene extracts. Evaporate the combined extracts to dryness at <40°C on a rotary evaporator.

HPLC Analysis

DPX-F5384 is determined by high performance liquid chromatography using a photoconductivity 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. The preparation of HPLC mobile phase, HPLC column cleaning solution, and DPX-F5384 standards is described in the "Apparatus and Reagents" section.

Care should be taken to properly condition and equilibrate the HPLC column before analysis. If the column is not properly conditioned, low sensitivity or rapidly drifting sensitivity may be experienced. Condition the column by pumping the HPLC cleaning solution through the column and detector at 0.5 mL/min for at least four hours but preferably overnight. 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 samples in 5 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:	35°C
Mobile Phase:	Composition given in "Apparatus and Reagent" section
Mobile Phase Flow-Rate:	1.0 mL/min
Injection Volume:	10 µL
Detector:	Tracor Model 965 photoconductivity
Retention Volume:	7.4 mL

At least one unfortified control sample and one control sample fortified with 0.50, 1.0, or 2.0 µg DPX-F5384 snould be prepared with each batch of samples and analyzed at random intervals or at predetermined uniform intervals during a series of

sample analyses. This will demonstrate the absence of interferences, provide a check on recovery efficiency, and confirm that the retention time in the sample matrix is consistent with that of the standards.

Calculations

Standard curves of DPX-F5384 peak height versus ng DPX-F5384 injected are linear with zero intercept for injections of at least 10 ng DPX-F5384 when analyzed under carefully controlled conditions. However, under typical sample analysis conditions, some drifting of the detector sensitivity is generally observed. This is conveniently accounted for by analyzing samples and standards alternately. Any drift in sensitivity can thus be readily monitored.

Calculate ppm DPX-F5384 in rice by using Equation 1. Calculate percent recovery for fortified control samples by using Equation 2.

$$\text{PPM DPX-F5384} = \frac{\text{PK} \times \text{ATTEN} \times \text{VS}}{\text{Mo} \times \text{VI} \times \text{SW}} \qquad (1)$$

$$\text{Percent Recovery} = \frac{100 \times \text{PK} \times \text{ATTEN} \times \text{VS}}{\text{Mo} \times \text{VI} \times \text{SP}} \qquad (2)$$

Variables in the above equations are defined as follows:

ATTEN = Detector attenuation setting

C = Concentration of DPX-F5384 standard in µg/mL

$$Mo = \frac{PK \times ATTEN}{VI \times C} = \text{Detector sensitivity in mm/µg injected}$$

PK = DPX-F5384 peak height in mm

SP = Weight of DPX-F5384 fortified onto sample in µg

SW = Weight of sample in g

VI = Volume of sample solution injected in mL

VS = Volume of sample solution in mL

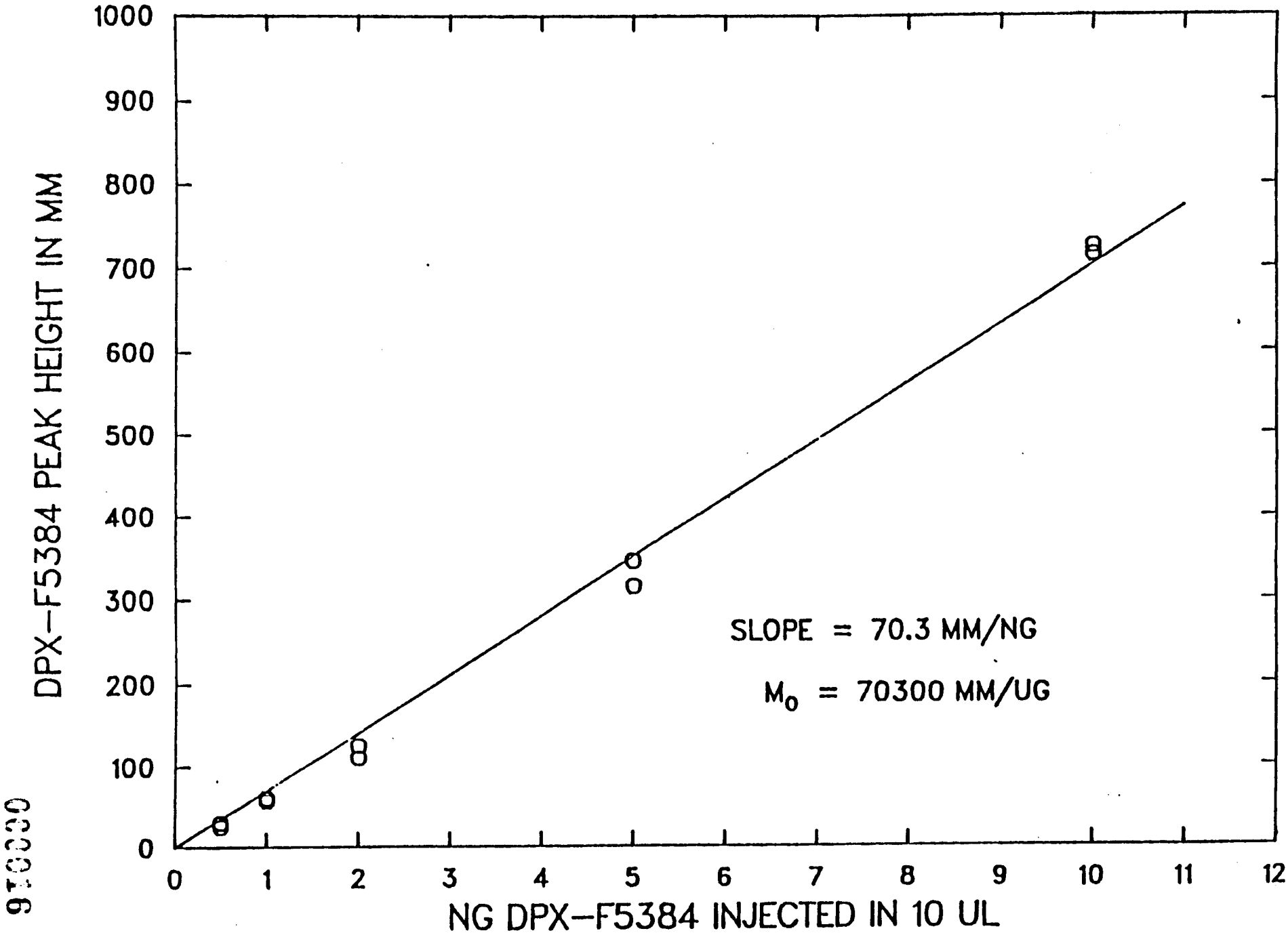
DISCUSSION

A standard curve for HPLC analysis of DPX-F5384 using a photoconductivity detector is shown in Figure 1. Chromatograms for determination of DPX-F5384 in a rice control sample and in rice control samples fortified before analysis with DPX-F5384 at 0.02, 0.04, and 0.08 ppm are shown in Figure 2.

Recovery efficiency was determined during routine analyses of rough rice samples by fortifying known quantities of DPX-F5384 onto control samples at 0.02 to 0.08 ppm and then determining DPX-F5384 in the fortified samples by the analytical method described here. The recovery efficiency of the method is 96% (N = 16, Std Dev = 8.0%).

The detection limit of the method is 0.02 ppm. The detection limit is determined primarily by the small interfering peak in control samples. The detection limit and recovery efficiency are adequate for the evaluation of DPX-F5384 residues in rice.

FIGURE 1 STANDARD CURVE FOR DPX-F5384



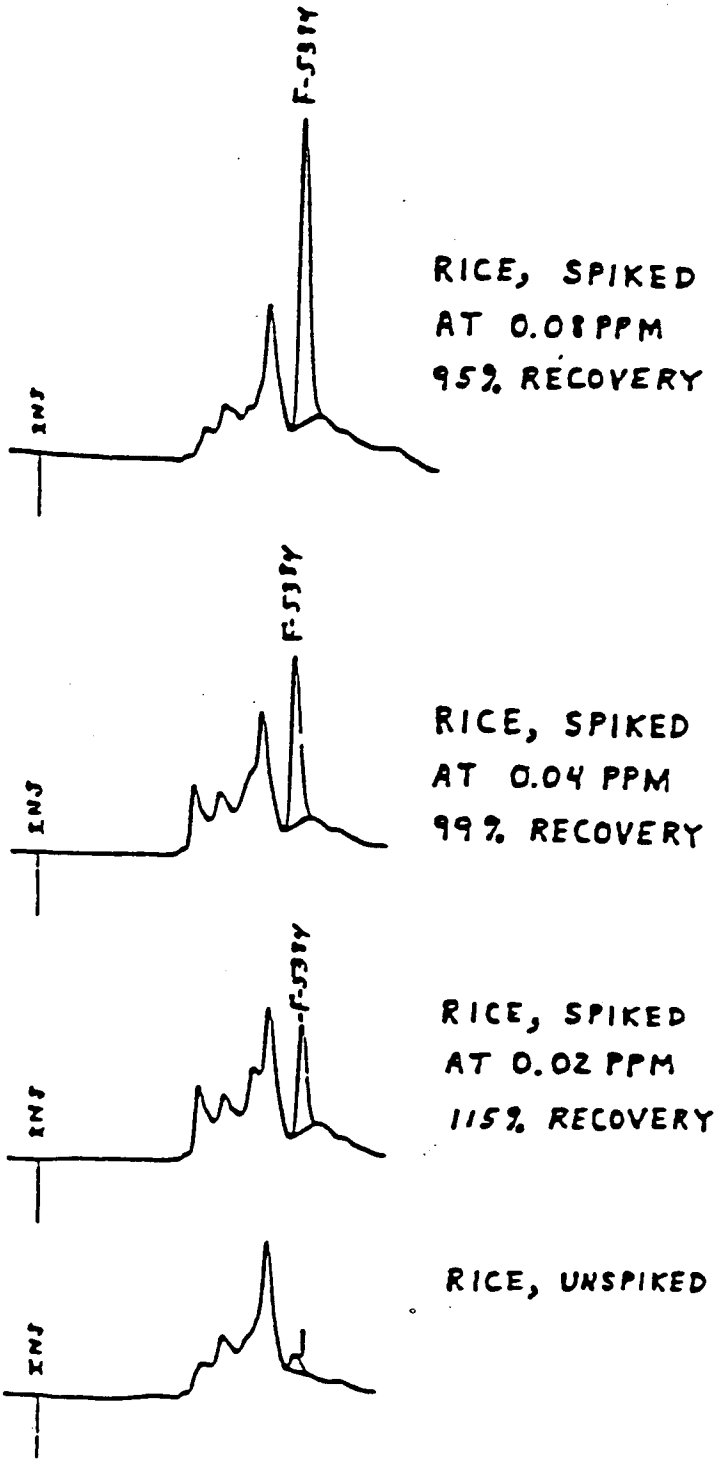


FIGURE 2 CHROMATOGRAMS FOR DETERMINATION OF DPX-F5384 IN POLISHED RICE GRAIN