Analytical Method for the Quantitation of DPX-E9636 in Soil by Liquid Chromatography

Jennifer S. Amoo

Revisions Made to the Original Report

Reasons for the Revisions and Impact on the Method

The original analytical method developed included a clean-up step utilizing carbon tetrachloride. Due to a German Decree for the protection of the user, non-official institutions are not allowed to handle carbon tetrachloride. Therefore the analytical method had to be modified to eliminate the use of carbon tetrachloride. This has been accomplished with the incorporation of centrifugation and filtration steps. This revised method was successfully validated in three German soils.

LOCATION*	REVISION
Page 1	Inclusion of Data Requirement
Page 9	Principle paragraph describes the revised extraction procedure. In the revised method, the carbon tetrachloride extraction step was eliminated. The sample residue after evaporation was instead dissolved in an aliquot of the pH 7 buffer, chilled, acidified, centrifuged, and filtered through a 0.45-µm filter unit before injection into the HPLC system for analysis. The revised extraction steps are incorporated under the Extraction Procedure paragraph.
Page 11	The HPLC system used for the revised method has since been upgraded to include a Waters Model 486 UV detector and the Waters Millenium 2010 Chromatography Manager WorkStation replacing the original Waters Model 481

* Revisions located in this report.

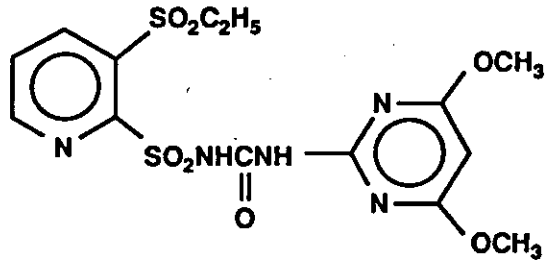
LOCATION	REVISION
	detector and Waters Model 745B Data Module.
Pages 12-13	New equipment and reagents used in the revised method are listed.
Page 14	The Standard Spiking Solution concentration used in the revised method was 1.0 µg/mL instead of 0.1 µg/mL.
Pages 14-16	The extraction procedure incorporates the revised method with the elimination of carbon tetrachloride from the procedure.
Page 17	Chromatographic standards were prepared with the new Standard Spiking Solution at concentrations of 0.002 µg/mL to 0.030 µg/mL.
Pages 17-20	In the Operating Conditions paragraph, the changes in the valve-switching event times and the Pump gradient table are shown and discussed to reflect the modifications to the HPLC system since the original method.
Pages 18, 22	The column-switching window time was changed from 5.5 minutes to 5 minutes.
Pages 24-26	Changes to reflect the new accuracy, precision, and LOD values for the revised method.
Pages 28-29	Three German soils were used for the revised method validation. Two of them were the same ones used in the original method and the Tama soil was replaced by a third German soil.
Pages 34-48	Figures 4-12 are replaced with chromatograms from the revised method.

INTRODUCTION

A. *Scope*

An analytical method based on the use of a liquid chromatograph utilizing eluent and column switching with photometric detection at 254 nm is described for the determination of DPX-E9636 in soil at levels as low as 0.20 ppb (limit of quantitation) using a 200-gram sample.

DPX-E9636 is a sulfonylurea herbicide whose structure is shown below:



DPX-E9636

N((4,6-dimethoxy-pyrimidin-2-yl)aminocarbonyl)-3-(ethylsulfonyl)-2-pyridinesulfonamide.

B. *Principle*

Four 50-gram soil sample aliquots are extracted with 50 mL of a 1:1 solvent mixture of acetonitrile and methylene chloride. The extracts are combined and evaporated to near dryness. The residue is diluted with a pH 7 phosphate solution. This solution is then chilled, acidified with 10 µL of concentrated phosphoric acid to a pH of 2.5-3.5, centrifuged, and filtered.

Two milliliters of this sample extract are injected onto a phenyl HPLC clean-up column and chromatographed in an aqueous eluent of 43% methanol buffered at pH 3.5. At this pH, DPX-E9636 is predominantly uncharged and thus concentrates at the head of the phenyl column during injection and, as chromatography continues, it separates from a large number of polar compounds which could interfere in the subsequent analytical step. At a predetermined time, the

eluent is switched to one of lower methanol concentration (10%) and higher pH (7.5). Most potential interferences which would have eluted with the analyte in the first eluent are now eliminated as they become highly retained by the column. DPX-E9636 continues to elute close to its original rate despite the lowered methanol concentration because it is anionic at pH 7.5 and very weakly retained by the phenyl column. Shortly before the analyte would elute on the phenyl column, the eluent containing the DPX-E9636 peak is switched to an Rx-C_8 analytical column. Following transfer of the peak, chromatography continues on this second column only, in an eluent of 20% methanol buffered at pH 7.0. DPX-E9636 is detected with a UV detector at 254 nm. This chromatographic system is similar to the prototype column/eluent switching method for sulfonylureas developed by J. H. Larochelle, et al. (Reference 1).

MATERIALS/METHODS

A. *Equipment*

Liquid Chromatograph

System Controller, Waters Model 600E
(Millipore Inc., Milford, Mass.)

Multisolvent Delivery System, Waters Model 600E
(Millipore Inc., Milford, Mass.)

Autoinjector and Cooling System, Waters Model 712 WISP (Millipore Inc., Milford, Mass.)

Waters Automated Valve Station (WAVS)
(Millipore Inc., Milford, Mass.)

Waters Millenium 2010 Chromatography Manager
(Millipore Inc., Milford, Mass.)

Column Heater and Temperature Control Module,
(Millipore Inc., Milford, Mass.)

Waters Model 486 UV Detector
(Millipore Inc., Milford, Mass.)

Pump, Eldex Model A-30-S
(Eldex Laboratories Inc., San Carlos, Calif.)

HPLC Columns

Column 1: Du Pont Zorbax® Phenyl 4.0 x 80 mm, 5-µm Reliance Cartridge, #820662-942 (MAC-MOD Analytical Inc., Chadds Ford, Pa.)

Column 2: Du Pont Zorbax® Rx-C_8 4.6 x 250 mm, 5-µm Analytical Column, #880967-901 (MAC-MOD Analytical Inc., Chadds Ford, Pa.)

Mechanical Wrist-Action Shaker: Burrell Model 75 Shaker (Burrell Corporation, Pittsburgh, Pa.)

Rotary Evaporator: Buchi Model RE111 Rotavapor and Evapotec Thermo-lift Temperator Control Water Bath (Brinkman Instruments Co., Westbury, NY, and Haake Buchler Instruments, Inc., Saddle Brook, N.J.)

Centrifuge: Du Pont Sorvall® Model RC-5C refrigerated centrifuge (Du Pont Instruments, Wilmington, Del.)

Centrifuge Rotors: Du Pont Models HS4 and SS34 (Du Pont Instruments, Wilmington, Del.)

Balance: Mettler BB300 balance (Mettler Instruments Co., Hightstown, N.J.)

Centrifuge Bottles: 250-mL polypropylene, Nalge #21020-028 (VWR Scientific, Bridgeport, N.J.)

Round Bottom Flasks: 500 mL, KIMAX® boiling flasks (VWR Scientific)

Narrow-range pH Paper: EM ColorpHast® Indicator strips, narrow range pH 0-6 (VWR Scientific)

pH Meter: Beckman Model PHI 11 (Beckman Instruments, Inc., Fullerton, Calif.)

Syringes: 5-mL plastic disposable syringes with luer connection (VWR Scientific)

Millex-HV 0.45 µM Filter Units, Cat. No. SLHV025NS (Millipore Corporation, Bedford, MA)

Corning Graduated Polypropylene Centrifuge Tubes, 15 mL with plug seal caps, #25319-15 (Corning Inc., Corning, NY)

B. ***Reagents and Standards***

Water - Deionized water passed through a Milli-Q® Water Purification System (Millipore Corp., Milford, Mass.)

Methanol - "EM Omnisolv" #MX0488-1 (EM Science, Cherry Hill, N.J.)

Acetonitrile - "EM Omnisolv" #AX0142-1 (EM Science, Cherry Hill, N.J.)

Methylene Chloride - "EM Omnisolv" #DX0831-1 (EM Science, Cherry Hill, N.J.)

K_2HPO_4 - "Baker Analyzed Reagent #3252-01 (J. T. Baker Chemical Co., Phillipsburg, N.J.)

KH_2PO_4 - "EM low absorbance grade #PX 1566-2 (EM Science, Cherry Hill, N.J.)

85% Phosphoric acid - Fisher o-phosphoric acid, 85%, HPLC grade #A260-500 (Fisher Scientific Co.)

DPX-E9636 - DPX-E9636-43 Reference Standard, Purity of 99.68% (Du Pont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, Del.)

C. ***Preparation of Solutions***

1M KH_2PO_4:

Dissolve 136 g of KH_2PO_4 in 800 mL of water and dilute to 1 liter. Filter through a 0.22-µm filter.

1M K_2HPO_4:

Dissolve 174 g of K_2HPO_4 in 800 mL of water and dilute to 1 liter. Filter as above.

10 mM K_2HPO_4 Solution, pH 7.0:

Add 10 mL of 1M K_2HPO_4 to 1000 mL of deionized water. Adjust pH to 7.0 with conc. phosphoric acid (85%).

Extraction Solution:

50% Acetonitrile/50% Methylene Chloride
Mix 1000 mL of acetonitrile and 1000-mL methylene chloride.

Chromatographic Eluents

Eluent A, 43% Methanol/57% 0.03 M Potassium Phosphate, pH 3.5

Add 60 mL of 1 M KH_2PO_4 to a 2000-mL solvent bottle and dilute with 1080 mL of deionized water. Add 860 mL of methanol and mix. Using a calibrated pH meter, adjust pH of this solution to 3.5 by addition of concentrated phosphoric acid.

Eluent B, 10% Methanol/90% 0.01 M Potassium Phosphate, pH 7.5

Add 20 mL of 1 M K_2HPO_4 to a 2000-mL solvent bottle and dilute with 1780 mL of deionized water. Add 200 mL of methanol and mix. Using a calibrated pH meter, adjust pH of this solution to 7.5 by addition of concentrated phosphoric acid.

Eluent C, 20% Methanol/80% 0.01 M Potassium Phosphate, pH 7.0

Add 20 mL of 1 M K_2HPO_4 to a 2000-mL solvent bottle and dilute with 1580 mL of deionized water. Add 400 mL of methanol and mix. Using a calibrated pH meter, adjust pH of this solution to 7.0 by addition of concentrated phosphoric acid.

Eluent D, 90% Methanol/10% Water

Add 200 mL of water to 1800 mL of methanol.

Standards

Stock Standard Solution:

Accurately weigh out 10 (± 0.01) mg of DPX-E9636-43 and dissolve in 100 mL of acetonitrile to make a 100-µg/mL stock standard. This standard is stable for at least six months if stored at 4°C.

Standard Spiking Solution:

Prepare a 1.00-µg/mL spiking solution by adding 1000 µL of 100-µg/mL Stock Standard Solution to a 100-mL volumetric flask and diluting to 100 mL with acetonitrile.

This solution may be refrigerated for at least 3-4 weeks. This spiking solution is used for fortification of samples and preparation of chromatographic standards.

D. Extraction Procedure

Preliminary Treatment

Soil samples are homogenized in the presence of dry ice using a Hobart food chopper. Homogenized soil samples are weighed out into 50-gram aliquots and placed in 250-mL centrifuge bottles, capped, and stored in a freezer at -20°C until fortification and extraction.

Fortification

Fortify each set of four 50-g samples with an appropriate amount of the 1.00-µg/mL Spiking Solution. Allow the acetonitrile to evaporate for about 15 minutes. For method

validation, samples were fortified at levels of 0.200 ppb, 0.500 ppb, and 1.000 ppb as follows:

For each set of four 50-g samples, to prepare:

0.200-ppb fortification, add 10 µL of 1.00-µg/mL Spiking Solution to each 50-g soil aliquot.

0.500-ppb fortification, add 25 µL of 1.00-µg/mL Spiking Solution to each 50-g soil aliquot.

1.00-ppb fortification, add 50 µL of 1.00-µg/mL Spiking Solution to each 50-g soil aliquot.

Extraction (For each 200-g sample)

1. Weigh out four 50-gram (± 0.1) aliquots of soil into tared 250-mL centrifuge bottles.
2. Fortify as in the Fortification step if required.
3. Add 5 mL of deionized water to each 50-gram soil aliquot to enhance recovery of DPX-E9636. This amount should be enough to just wet the soil aliquot.
4. Add 50 (± 2) mL of 50% acetonitrile/50% methylene chloride mixture to each bottle; cap each bottle. (Teflon® tape rims of centrifuge bottles to ensure against leakage.)
5. Shake bottles for 10 minutes on a mechanical wrist-action shaker.
6. Centrifuge the sample-solvent mixture in the Sorvall® RC-5C centrifuge (HS-rotor) for 10 minutes at 3000 rpm at 0°C. If using an alternate speed, centrifuge until most particulates have separated from the solvent mixture.
7. Decant and combine the extracts from the four 50-g soil aliquots into a 500-mL round bottom flask.
8. Repeat Steps 4-7. (Make sure soil is re-dispersed in second aliquot of 50% acetonitrile/50% methylene chloride.)

   Decant and combine these 4 extracts with the extract in the round-bottom flask. So, for each 200-g soil sample, four 50-g fractions of soil are extracted with a total of approximately 400 mL of 50% acetonitrile/50% methylene chloride and combined to yield a single sample extract.

9. Evaporate each combined soil extract (~400 mL) to approximately 2 mL (DO NOT LET GO TO DRYNESS) on the Rotovapor in a water bath set at 40°C.
10. Transfer the residue to a 15-mL graduated centrifuge tube.
11. Add approximately 5 mL of 10 mM phosphate solution at pH 7.0 to the round-bottom flask to rinse and transfer the solution to the centrifuge tube.
12. Bring the final volume in the tube to 10 mL with the 10 mM phosphate buffer, pH 7.0. Cap and vortex.

    The extraction can be interrupted at this stage if required. Samples are stable enough at this stage for overnight, freezer storage.
13. Chill the sample in the tube at 0°C for 30 minutes.
14. Add 10 µL of concentrated phosphoric acid to the centrifuge tube to acidify to pH 2.5-3.5. Vortex.
15. Chill the sample in the tube at 0°C for 15 minutes.
16. Centrifuge the sample tube at 3000 rpm at 0°C for 10 minutes.
17. Filter the sample through a 0.45-µm filter unit (pre-wetted with the pH 7 phosphate solution or deionized water) using a 5-mL disposable syringe into a 4-mL sample vial.
18. Place in the autoinjector cooled to 0°C to -5°C.
19. Inject 2 mL of the acidified extract.

E. *Chromatographic Procedure*

The chromatographic system design is shown in Figure 1. At low pH, DPX-E9636 is uncharged and relatively non-polar. Two mL of the acidified sample extract are injected onto a phenyl HPLC column (Column 1) and chromatographed in an aqueous eluent of 43% methanol buffered at pH 3.5 (Eluent A). DPX-E9636 concentrates at the head of the column at injection and as chromatography continues, the eluent is switched to one of lower methanol concentration and higher pH (Eluent B). At this higher pH, DPX-E9636 is negatively charged and consequently more polar and will rapidly elute from the column. Shortly before the DPX-E9636 would elute from the phenyl column (Column 1), it is automatically transferred to the Rx-C_8 analytical column (Column 2) where it again

concentrates at the column head. Chromatography continues on the second column only, in an aqueous eluent of 20% methanol buffered at pH 7.0 (Eluent C) until the DPX-E9636 is eluted and detected by a UV detector set at 254 nm. Eluent D, 90% methanol/10% water, is used to clean both columns, Column 1 by backflushing with pump 2 during the run and Column 2 by flushing with pump 1 after the run. Finally, both columns are re-equilibrated with the appropriate eluents.

Chromatographic Standard Solutions

Chromatographic standards ranging from 0.002 µg/mL to 0.030 µg/mL are prepared by pipetting 20 µL, 40 µL, 100 µL, and 300 µL of the 1.00-µg/mL spiking solution into 15-mL centrifuge tubes and then diluting with 10 mL of 10 mM phosphate solution, pH 7.0.

Standard Concentration (µg/mL)	Volume of 1.00 µg/mL Stock Standard Solution (µL)	Final Volume (mL)
0.002	20	10
0.004	40	10
0.010	100	10
0.030	300	10

All chromatographic standards are adjusted to pH 2.5-3.5 with 10-µL concentrated phosphoric acid (85%) immediately before placing in the autoinjector for the start of chromatographic analysis.

Operating Conditions

See Table I for a list of chromatographic operating conditions.

a) Evaluation of the phenyl column (to be done daily):

The phenyl column used for this method is relatively inexpensive. It carries the burden of sample clean-up, and consequently has a limited life-time. Degradation of the column is evidenced by poor-peak shape and extreme tailing. The column must be evaluated daily to ensure adequate performance. Tailing is considered unacceptable when the peak width at baseline, including the tail, is more than about

4 minutes when a 0.030-µg/mL DPX-E9636 standard solution is chromatographed on the phenyl column alone with Eluent A only. Typically, the phenyl column must be replaced following 25 to 35 chromatographic runs (25 to 30 samples). When a new column is installed, it must be conditioned with Eluents A and B.

b) Establish the eluent switching time, T-B (to be done daily):

Equilibrate the Rx-C8 analytical column (Column 2) with Eluent C and equilibrate the phenyl column (Column 1) with Eluent A. Determine the retention time, RT1, of a 0.030-µg/mL DPX-E9636 standard on Column 1 with Eluent A only (about 18-22 minutes after chromatography begins). T-B, the time at which Eluent B will be introduced, is RT1 divided by 2 minutes. This time, specific to our instrument, allows about 5 minutes between the Eluent B solvent front and the analyte peak on the chromatogram. The actual time must be determined experimentally for an alternate instrument (see Section I).

c) Establish the column-switching time, T-S and T-E (to be done daily):

Set the T-B time and repeat chromatography of the 0.030-µg/mL standard on Column 1 only with a switch from Eluent A to Eluent B at the prescribed time, T-B. Determine the retention time, RT2, of the DPX-E9636 peak. The column-switching times, T-S (start of the column-switching window), and T-E (end of the column-switching window) are set as RT2 minus 2 minutes and plus 3 minutes, respectively. This permits a 5.0-minute window during which the phenyl column effluent is transfered directly to the Rx-C_8 analytical column. The time of switching to Eluent C is set at the T-S time so that the new eluent is introduced after column-switching has taken place thus preventing build-up of back pressure in the system due to mixing eluents. A table of the Pump Eluent-switching and Valve-switching programs are shown below:

Chromatographic Programming

Valve-Switching Events

	Time (min)	Event*	Action	Comments
	Initial	S1	OFF	Column 1 on-line
	Initial	S2	OFF	Column 2 off-line
	Initial	S3	OFF	Eluent A on-line
(T-S)	19.73	S2	ON	Column 2 on-line with 1
(T-E)	24.73	S1	ON	Column 1 off-line
	24.93	S3	ON	Backflush Col. 1 w/pump 2

Flushing and Re-equilibration of Columns 1 and 2

63.00	S3	OFF	Stop backflush
63.00	S2	OFF	Column 2 off-line
63.00	S1	OFF	Column 1 on-line in Eluent A

* S1 - Switch valve that controls Column 1
S2 - Switch valve that controls Column 2
S3 - Switch valve that controls the BackFlush Pump

Pump Gradient Program

		Solvent Channels			
Time (min)	Flow (mL/min)	% A	% B	% C	% D
Initial[1]	1.50	100	0	0	0
10.00[2] (T-B)	1.50	0	100	0	0
19.73[3] (T-S)	1.50	0	0	100	0
45.00[4]	1.50	0	0	0	100
51.00[5]	1.50	0	0	100	0
63.00[6]	1.50	100	0	0	0
75.00[7]	1.50	100	0	0	0

[1] Sample injected onto Column 1 and chromatographed in Eluent A to detector.

[2] Begin elution of DPX-E9636 from Column 1 by change to high pH eluent.

[3] Chromatograph analyte in Column 2 in higher methanol eluent.

[4] Clean Column 2 with 90% methanol.

[5] Re-equilibrate Column 2 in high pH/low methanol eluent for next run.

[6] Re-equilibrate Column 1 in high methanol/low pH eluent for next run.

[7] Next sample injected.

d) Chromatography of samples and standards:

Set T-B, T-S, and T-E times as determined above. Chromatograph prepared standards and samples.

Calibration Procedure

Prepare DPX-E9636 chromatographic standard solutions at 0.002 µg/mL, 0.004 µg/mL, 0.010 µg/mL, and 0.030 µg/mL.

Chromatographic standards must be chilled and acidified before chromatography the same way as the samples. Chromatograph each standard solution. DPX-E9636 has a retention time of 37-39 minutes.

Measure the DPX-E9636 peak area from the chromatogram. A plot of peak area versus concentration should be linear and pass through the origin.

Determination of DPX-E9636 in Samples

Chromatograph each acidified, chilled sample extract. Identify the presence or absence of DPX-E9636 based on its retention time determined in the standard runs. Record the DPX-E9636 peak areas.

Calculate the concentration of DPX-E9636 in each sample using the equation described under Method of Calculations.

F. *Interferences*

Several sulfonylurea residue methods (References 2, 3) have been developed using the same technique and have been found to be very specific and relatively free of significant interference. We expect this method to be free of interferences from other pesticides and impurities found in reagents, sample matrices, and on glassware, as we do not expect to find compounds of the same acid-base character.

If a significant interference is apparent, the pH of Eluent C may be adjusted slightly to selectively move the DPX-E9636 peak relative to the interference. An adjustment in pH of 0.2 pH units or less in either direction is suggested.

G. Confirmatory Techniques

The acid/base character of DPX-E9636 may be used for confirmation. Changing the pH of Eluent C by 0.2 to 0.5 pH units will selectively change the retention time of the DPX-E9636 peak. (Do not exceed a pH of 7.5 to avoid column degradation.) If the pH is raised, the methanol concentration may need to be decreased to obtain the desired retention. Similarly, a decrease in eluent pH may require an increase in methanol concentration to maintain a reasonable retention time.

The presence of DPX-E9636 may also be confirmed by substituting an Rx-C_{18} column or Zorbax® Phenyl analytical column for the Zorbax® Rx-C_8 column and slightly increasing the methanol concentration in Eluent C to maintain the retention time of DPX-E9636.

H. Time Required for Analysis

Typically, 10 samples can be prepared in 4-6 hours. Time for chromatography is 75 minutes per sample or standard. Determination of eluent and column-switching times can be performed during sample preparation. However, samples must be run within a couple of hours after acidification, due to the rapid decomposition of DPX-E9636, limiting the number of samples prepared to about 8 per day. Samples must be kept at a temperature of 0°C to -5°C at all times to slow down DPX-E9636 decomposition.

I. Modifications and Potential Problems

To ensure against leakage of extract, the rims of the centrifuge bottles may be coated with Teflon® tape which offers a tight closure when capped. Care must be taken when transferring extracts from the round bottom flask to the centrifuge tubes. The walls of the flask must be swirled and rinsed thoroughly with the pH 7 phosphate buffer aliquots and then poured into the tube to ensure quantitative transfer of DPX-E9636 in the extract.

A known aliquot of deionized water may be added to soil fractions prior to fortification and extraction to enhance the extraction of the analyte from the soil.

When using alternate equipment, the actual arrival time of a new eluent to a column will depend on the dead volume between the pump and the column. Thus, differences in pump head and tubing volumes for different chromatographs will change the time for eluent arrival to the column. The RT1 time may have to be adjusted for the particular chromatograph. It should be selected such that the DPX-E9636 peak is sufficiently resolved from the solvent front on the chromatogram, and such that co-eluting sample components will not interfere with the DPX-E9636 peak on the second column. Additional time may also be required for cleaning and re-equilibration of Column 2 to allow for longer eluent arrival times. If a different autosampler or autoinjector is inserted into the HPLC system between the pump and column, pre-column volume may be further increased, affecting eluent switching times still more.

The column-switching time window, as defined by T-S and T-E, is set to last 5.0 minutes and is skewed relative to the DPX-E9636 peak (retention time minus 2 minutes to retention time plus 3 minutes). Shorter or longer column-switching windows may be possible depending on the instrument, column, and flow-rate of the eluent used. The window length must be adequate to encompass the analyte peak and also should allow for drift and column degradation (as evidenced by peak tailing) on the first column. If resolution is insufficient on the Zorbax® Rx-C_8 analytical column, Eluent C may be altered by changing its pH and/or methanol concentration. A change in pH should selectively move the DPX-E9636 peak relative to sample matrix components; the methanol concentration may then be adjusted to achieve suitable retention times.

J. Method of Calculation

1. Calibration Factor (CF)

The Calibration Factor (CF) is the ratio of DPX-E9636 detector response (peak height or area) to DPX-E9636 concentration. For this method, peak areas were used. The CF for each chromatographic standard run was calculated. A standard is typically run before and after every 2 samples. The average of CFs from the standard runs preceding and following a set of samples is used for calculation of DPX-E9636

concentrations in those samples.

$$CF = \frac{\text{Peak Height of DPX-E9636 Standard}}{\text{Concentration (µg/mL) of DPX-E9636 Standard}}$$

Average Calibration Factor (Avg. CF):

$$\text{Avg. CF} = \frac{\text{Sum of Calibration Factors}}{\text{No. of DPX-E9636 Standards}}$$

2. *Concentration of Analyte in Sample (ppb)*

$$\text{ng/g (ppb) DPX-E9636 in sample} = \frac{A \times FV \times 1000}{\text{Avg CF} \times SW}$$

where:

A = Peak area of DPX-E9636

FV = Final volume of extract. Ten mL of extract is the final volume for all samples and standards.

SW = Sample weight in grams, 200 grams

Avg. CF = Average Calibration factor for two standards bracketing sample set of 2 samples.

3. *% Recovery*

$$\% \text{ Recovery} = \frac{\text{Amount recovered (ppb)}}{\text{Fortification level (ppb)}} \times 100$$

CERTIFICATION

ANALYTICAL METHOD FOR THE QUANTITATION OF DPX-E9636 IN SOIL BY LIQUID CHROMATOGRAPHY

We, the undersigned, declare that the work described in this revision was performed under our supervision, and that this report provides an accurate record of the procedures and results.

Report by:

Jennifer S. Amoo — October 18, 1993
Jennifer S. Amoo — Date
Study Director

Approved by:

Richard F. Sauers — October 18, 1993
Richard F. Sauers — Date
Research Supervisor

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Notebook References:

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E. I. du Pont de Nemours and Company
Du Pont Agricultural Products
Experimental Station
Wilmington, Delaware 19880-0402
and/or
Du Pont Records Management Center
Wilmington, Delaware 19880-0870

Sponsor:

E. I. du Pont de Nemours and Company
Du Pont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, Delaware 19880-0402

TABLE I
SUMMARY OF CHROMATOGRAPHIC CONDITIONS

Column:	1	Zorbax® Phenyl 4.0 mm x 80 mm, 5-micron Reliance series cartridge
	2	Zorbax® Rx-C_8 4.6 mm x 250 mm, 5-micron Analytical
Column Temperature:		35°C
Flow Rate:		1.5 mL/min
Eluent:	A	30 mM KH_2PO_4 in 43% methanol, pH 3.5
	B	10 mM K_2HPO_4 in 10% methanol, pH 7.5
	C	10 mM K_2HPO_4 in 20% methanol, pH 7.0
	D	90% methanol/10% water
Injection Volume:		2.0 mL
Detector:	Wavelength	254 nm
	Sensitivity	1 Volt = 1 AUFS