Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Indoxacarb (DPX-KN128)

MRID #: 444773-16

Matrix: Soil

Analysis: GC/MS

This method is provided to you by the Environmental Protection Agency's (EPA) Environmental Chemistry Laboratory (ECL). This method *is not* an EPA method but one which was submitted to EPA by the pesticide manufacturer to support product registration. EPA recognizes that the methods may be of some utility to state, tribal, and local authorities, but makes no claim of validity by posting these methods. Although the Agency reviews *all* Environmental Chemistry Methods submitted in support of pesticide registration, the ECL evaluates only about 30% of the currently available methods. Most methods perform satisfactorily but some, particularly the older methods, have deficiencies. Moreover, the print quality of the methods varies considerably because the methods originate from different sources. Therefore, the methods offered represent the best available copies.

If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

444773-16

Study Title

ENVIRONMENTAL CHEMISTRY METHOD FOR THE DETERMINATION OF DPX-KN128 RESIDUES IN SOIL USING GC-MSD

Test Guideline

EEC Directive 91/414/EEC: Annex II 4.2.2

U.S. EPA Pesticide Assessment Guidelines
Subdivision N, Series 164, 165, & 166

Authors of Original Report And Revision No. 1

Gary L. Westberg
Daniel R. Vincent

Date Study Completed

Original Report: June 18, 1997
Revision No. 1: November 10, 1997

Performing Laboratory

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

Laboratory Project ID

AMR 4367-97
Revision No. 1

STATEMENT OF NO DATA CONFIDENTIALITY

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C).

Company

E. I. du Pont de Nemours and Company

Company Agent

Thomas P. Price
(Typed Name)

U. S. Product Registration Manager
(Title)

Patricia G. Devine for Thomas P. Price
(Signature)

November 25, 1997
(Date)

2

GOOD LABORATORY PRACTICE STATEMENT

The EPA Good Laboratory Practice (GLP) requirements specified in 40 CFR Part 160 do not require analytical methods to be developed under Good Laboratory Practices (GLP). However, the methods development presented in this revision was done under GLP except that no protocol was written, no conduct audit was performed, and no QA audit of the study records was done. Analytical procedures, documentation, and archiving of the validation data were done by Standard Operating Procedures.

Sponsor:

E. I. du Pont de Nemours and Company

Submitter:

E. I. du Pont de Nemours and Company

Study Director

D. Vincent

Daniel R. Vincent, Ph.D.
Senior Research Biochemist

10 Nov 97

Date

Company Representative

Patricia G. Devine for
Thomas P. Price

DuPont Registration Representative

Nov. 25, 1997

Date

CERTIFICATION

ENVIRONMENTAL CHEMISTRY METHOD FOR THE DETERMINATION OF DPX-KN128 RESIDUES IN SOIL USING GC-MSD

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.

Revision No. 1 by:

Daniel R. Vincent — 10 November 1997
Study Director — Date

Approved by:

Sidney S. Goldberg — 10-Nov-97
Research Supervisor — Date

Date Study Initiated:
September 18, 1996 (first set of validation samples extracted)

Date Original Study Completed:
June 18, 1997

Date Revision No. 1 Completed:
November 10, 1997

Sponsor:
E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Meaning
µL	micro-liter (= 10^{-6} L)
ACN	acetonitrile
CAS	*Chemical Abstracts* Service
GC	gas chromatography
i.d.	inside diameter
L	liter
LOD	Limit of Detection (approximately 1/3 of the LOQ)
LOQ	Limit of Quantitation (5- to 20-times the noise)
m	meter
min	minute(s)
mL	milliliter (= 10^{-3} L)
mm	millimeter (10^{-3} m)
MSD	mass-selective detector
ppm	parts-per-million (equivalent to mg/kg)
rpm	revolutions-per-minute
sec	second(s)
SIM	single-ion monitoring, a mode in the MSD
SPE	solid-phase extraction

TABLE OF CONTENTS

BASIC STUDY INFORMATION

Study Number

AMR 4367-97

Study Title

Environmental Chemistry Method for the Determination of DPX-KN128 Residues in Soil Using GC-MSD

Purpose

This method was prepared to collect data and satisfy regulatory requirements.

Study Director

Daniel R. Vincent
E. I. du Pont de Nemours and Company
DuPont Agricultural Products
Global Technology Division
Experimental Station
Wilmington, DE 19880-0402

Study Category

Analytical Methods

Test Substance

DPX-KN128/IN-KN127

Study Execution Dates

September 18, 1996 (first samples extracted)
December 20, 1997 (last sample analyzed)

Testing Facilities

Morse Laboratories, Inc.
1525 Fulton Avenue
Sacramento, CA 95825

Study Personnel

Kimberly A. Tufts
Susan Clark
Kevin Clark

Related Documents

- Rhodes, B. C., 1997. "Aerobic Soil Metabolism of [^{14}C]DPX-JW62", DuPont Report No. AMR 2803-93, E. I. du Pont de Nemours and Company, DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
- Rühl, J. C., 1997. "Field Soil Dissipation of [^{14}C]DPX-JW062 (Racemic Mixture of DPX-KN128 and IN-KN127) Following Application of DPX-JW062 Experimental Insecticide.", DuPont Study No. AMR 3400-95, DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
- Vincent, D. R., McCooey, K. T., and Rühl, J. C., 1997. "Field Soil Dissipation of DPX-JW062 (Racemic Mixture of DPX-KN128 [Insecticidally Active Enantiomer] and IN-KN127) Following Application of DPX-JW062 Experimental Insecticide to Bare Ground", DuPont Report No. AMR 3402-95, DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
- D. R. Vincent, K. T. McCooey, 1997. "Field Dissipation of DPX-JW062 (Racemic Mixture of DPX-KN128 [Insecticidally Active Enantiomer] and IN-KN127) in Cabbage and Lettuce Following Application of DPX-JW062 Experimental Insecticide at Maximum Label Rates", DuPont Report No. AMR 3294-95, DuPont Agricultural Products, E. I. du Pont de Nemours and Company, Wilmington, DE.
- CAN 96-902, study in progress

ENVIRONMENTAL CHEMISTRY METHOD FOR THE DETERMINATION OF DPX-KN128 RESIDUES IN SOIL USING GC-MSD

Gary L. Westberg & Daniel R. Vincent

REASON FOR REVISION

This method was revised to remove the analysis of IN-KG433. Although this analyte was observed in laboratory soil metabolism studies, it has not been observed in field dissipation studies. Therefore, it is not necessary to include it as an analyte in soil analyses for either future soil studies or for environmental testing.

1.0 ABSTRACT

DPX-MP062 and DPX-JW062 Experimental Insecticides are formulated with an active ingredient that is a mixture of two enantiomers: DPX-KN128 and IN-KN127; DPX-KN128 is insecticidally active, while IN-KN127 is not. DPX-MP062 is comprised of approximately 3 parts of DPX-KN128 and approximately 1 part of IN-KN127. DPX-JW062 is a racemic mixture (50% of each) of the two enantiomers.

An environmental chemistry method has been established to determine residues of the insecticide DPX-KN128/IN-KN127 (parent) and its known metabolite DPX-JT333 in soil; enantiomers are not resolved in the analysis for either analyte. Soil samples are twice-extracted in acetonitrile (ACN) and water (9:1, v:v), with mixing energy supplied by a wrist-action shaker. Two phases are developed in the extract by adding solid sodium chloride. An aliquot of the ACN layer is removed from the salt-separated extract in order to analyze for parent and DPX-JT333. After evaporating the ACN, the sample is subjected to liquid:liquid partitioning and silica solid-phase extraction (SPE). Final extract is analyzed by single ion monitoring (SIM) GC-MSD. Parent and DPX-JT333 molecular ions are monitored in the analysis. Standards are prepared in control matrix extract to overcome observed matrix enhancement. The method has an apparent Limit of Quantitation (LOQ) of 0.01 ppm of parent equivalents from either analyte.

Recovery values for parent in a Canadian soil fortified at 0.010, 1.0, and 10 ppm were 105%, 96%, and 95%, respectively, with an overall average of 99%. Recovery values for DPX-JT333 in the same soil fortified at 0.010, 1.0, and 10 ppm were 97%, 94%, and 89%, respectively, with an overall average of 93%. The method was validated by measuring the recovery of each analyte in triplicate fortifications at three concentrations (0.010, 1.0, and 10 ppm). Two segments (0 to 15 cm and 30 to 45 cm) of a composite Canadian soil sample were used in the fortifications. The average recoveries are shown in the table below. The data indicate that the method is both accurate and precise.

Analyte	ppm Fortified	Percent Recovery
DPX-KN128/IN-KN127 (Parent)	0.010	105 ± 5
	1.0	96 ± 6
	10.	95 ± 14
DPX-JT333	0.010	97 ± 9
	1.0	94 ± 12
	10.	89 ± 11

2.0 INTRODUCTION

DPX-MP062 and DPX-JW062 Experimental Insecticides are being developed for control of various lepidopterous and other insect pests in cotton, sweet corn, pome fruits, vegetables, grapes, and root crops. The intended crop protection product will be used on a wide variety of crops, and the use will likely be multiple applications per season.

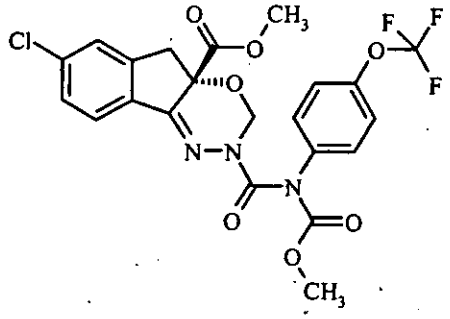
This environmental chemistry method is intended for the determination of DPX-KN128/IN-KN127 (parent) and its principal metabolite DPX-JT333 in soil samples. The principal use of the method has been to collect residue data for submission to regulatory authorities. Besides generating residue data, the method is intended to be used for regulatory surveillance.

DPX-KN128 is insecticidal, while its enantiomer, IN-KN127, is not. Since this chemical has a chiral center, the technical material exists as either a racemic mixture of both DPX-KN128 and IN-KN127 (DPX-JW062) or as an enhanced mixture containing approximately 3 parts of DPX-KN128 and 1 part of IN-KN127 (DPX-MP062). Known, significant, soil analytes include parent and DPX-JT333.

The analytical method does not resolve the enantiomers of either analyte. Concentrations of each analyte are measured, calculated, and reported as equivalents of either DPX-JW062 or DPX-MP062. In this method, parent refers to the starting reagent, whether it is DPX-JW062 or DPX-MP062; weights and concentrations are not adjusted for enantiomeric content. Parent and DPX-JT333 are detected as the particular molecular ion.

Structures, names and limited chemical data for the two analytes follow.

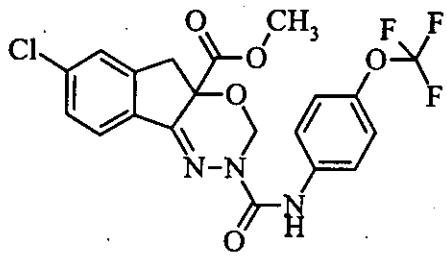
DPX-KN128



CAS Name (S)-methyl 7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylate

CAS Registry Number	173584-44-6
Molecular Weight	527.84
Molecular Composition	$C_{22}H_{17}ClF_3N_3O_7$

DPX-JT333



CAS Name	methyl 7-chloro-2,5-dihydro-2-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno[1,2-*e*][1,3,4]oxadiazine-4a(3*H*)-carboxylate
CAS Registry Number	144171-39-1
Molecular Weight	469.81
Molecular Composition	$C_{20}H_{15}ClF_3N_3O_5$

3.0 MATERIALS

Equivalent equipment and materials may be substituted unless otherwise specified; note any specifications in the following descriptions before making substitutions. Substitutions should only be made if equivalency/suitability has been verified with acceptable control and fortification recovery data.

3.1 Equipment

Assorted laboratory glassware including: graduated cylinders, short stem glass funnels, pipettes, volumetric flasks, evaporating flasks, microliter syringes are needed for this method.

Gas Chromatograph:	Hewlett-Packard 5890E gas chromatograph equipped with a Hewlett-Packard 5972 Mass Selective detector, an HP7673 Autosampler, and an HP G1034C MS ChemStation
GC Column:	10 m × 0.20 mm i.d. fused silica column crossbonded with a 0.33-µm film of DB-1 (J&W Scientific, Folsom, CA)
Balances:	Analytical balance capable of weighing to 0.1 mg for weighing analytical standards
	Top-loading balance capable of weighing to 0.01 g, for all other weighing

Centrifuge:	IEC Model HN-SII (Damon IEC Division, Needham Hts., MA)
Centrifuge Bottles:	250 mL, Nalgene® polyethylene bottles, with screw caps
Centrifuge Tubes:	50 mL, Pyrex® conical tubes, silanized, with Teflon®-lined screw caps
Evaporators:	N-Evap Laboratory Sample Evaporator Model 115 attached to a nitrogen source (Organomation Associates, South Berlin, MA)
Extraction Apparatus:	Wrist action shaker (Burrell Corporation, Pittsburgh, PA)
GC autosampler vials:	2.0 mL, glass, with 400-µL glass inserts (Alltech, Deerfield, IL)
Mixer:	Vortex Genie 2 (VWR Scientific, Bridgeport, NJ)
Separatory Funnels:	125 mL, Kimax, silanized, with Teflon® stopcocks
Solid-Phase Extraction Apparatus:	Vac Elut Model SPS 24 (Varian Analytical Instruments, Sunnyvale, CA)
Solid-Phase Extraction Columns:	Silica Bond Elut® Extraction Column, Part #1225-6026. 20 cc/5 g silica sorbent (Varian Analytical Instruments, Sunnyvale, CA) **Do not substitute.**
Syringe Needles:	4-inch, 13-gauge, stainless steel, Luer-Lok fitting (Popper & Sons, Inc., New Hyde Park, NY)
Syringes:	5 mL, glass, B&D Multifit, (VWR Scientific, Bridgeport, NJ)
Test Tubes:	13 × 100 mm, borosilicate, silanized, with Teflon®-lined screw caps. 16 × 150 mm, borosilicate, silanized
Assorted laboratory glassware	

3.2 Reagents and Standards

Acetonitrile:	Pesticide residue quality
Dimethyldichlorosilane:	Supelco, Catalog No. 3-3009 (Supelco, Inc., Bellefonte, PA)

Ethyl Acetate:	Pesticide residue quality
Hexane:	Pesticide residue quality
Isopropanol:	Pesticide residue quality
Toluene:	Pesticide residue quality
Sodium Chloride:	Reagent grade
Water:	HPLC grade
Silica wool:	Fused, catalog #20790 (Restek Corp., Bellefonte, PA)
DPX-KN128	Analytical standard grade DPX-KN128 (as either DPX-JW062 or DPX-MP062, available from DuPont Agricultural Products, Global Technology Division (E. I. du Pont de Nemours and Company, Wilmington, DE). The product used in this work was DPX-MP062-38, which had a purity of 97.1%.
DPX-JT333	Analytical standard grade DPX-JT333, available from DuPont Agricultural Products, Global Technology Division (E. I. du Pont de Nemours and Company, Wilmington, DE). The product used in this work was DPX-JT333-17, which had a purity of 98.3%.

3.3 Safety and Health

Each analyst must be acquainted with the potential hazards of the reagents, products and solvents used in this method before commencing laboratory work. All appropriate material safety data sheets should be read and followed, and proper personal protective equipment should be used.

4.0 METHODS

4.1 Principle of the Analytical Method

The soil sample is extracted twice with two volumes (2 mL/g of soil) of acetonitrile/water (9/1, v/v). The water and ACN in the extract are separated into two phases by adding solid sodium chloride. An aliquot of acetonitrile extract is then processed through additional analyte-specific cleanup procedures, including a hexane partition, a water/hexane partition, and a solid phase extraction (SPE) purification incorporating silica. Analytes are detected and quantified by gas chromatography using mass selective detection (MSD). The limit of quantitation for both analytes is 0.01 ppm.

4.2 Analytical Procedure

4.2.1 Glassware Cleaning and Preparation Procedures

The effectiveness of any cleaning procedure used should be demonstrated by preparation and analysis of reagent blanks. In general, all reusable glassware and plastic-ware should be washed in hot tap water with laboratory grade, non-phosphate detergent, rinsed several times with tap water, rinsed several times with de-ionized water, rinsed once with acetone, and allowed to dry before use. Care should be taken to avoid working with high levels of the analyte being monitored in the same laboratory where samples are being extracted and analyzed.

To avoid and/or reduce incidental adsorption of analytes to glassware, it is recommended that all glassware, except syringes, that contact sample extract be silanized. See Appendix 1 for detailed instructions on silanizing glassware.

4.2.2 Preparation of Reagent Solutions

2% Isopropanol/98% Hexane (v/v)
Add 20 mL isopropanol to 980-mL hexane. Mix.

5% Isopropanol/95% Hexane (v/v)
Add 50 mL isopropanol to 950-mL hexane. Mix.

5% Ethyl Acetate/95% Toluene (v/v)
Add 25 mL ethyl acetate to 475-mL toluene. Mix.

50% Ethyl Acetate/50% Hexane (v/v)
Add 500 mL ethyl acetate to 500-mL hexane. Mix.

10% Water/90% Acetonitrile (v/v)
Add 100 mL water to 900-mL acetonitrile. Mix.

4.2.3 Stock Standard Preparation and Stability

Weigh 12.5 mg (corrected for purity) of analytical standard and quantitatively transfer it to a 25-mL volumetric flask; separate solutions should be prepared for each analyte. Dissolve the standard in and bring to volume with ethyl acetate. The final concentration of the individual stock standard solutions is 500 µg/mL. Store the stock standard solutions at 1-8°C when not in use. Maximum storage life for the solution is 16 months for parent and 6 months for DPX-JT333.

4.2.4 *Fortification Standard Preparation and Storage*

Depending on the experimental design, prepare either separate or combined parent or DPX-JT333 standard solutions. Solutions at the following concentrations should be prepared every month.

100 µg/mL: Transfer 5.0 mL of 500-µg/mL stock standard solution(s) to a 25-mL volumetric flask. Bring to volume with ethyl acetate. Mix well. Store tightly capped at 1-8°C.

10 µg/mL: Transfer 2.5 mL of 100-µg/mL standard solution to a 25-mL volumetric flask. Bring to volume with ethyl acetate. Mix well. Store tightly capped at 1-8°C.

1 µg/mL: Transfer 2.5 mL of 10-µg/mL standard solution to a 25-mL volumetric flask. Bring to volume with ethyl acetate. Mix well. Store tightly capped at 1-8°C.

4.2.5 *Chromatographic Standard Preparation and Storage*

Prepare calibration standards in ethyl acetate. The following concentrations are needed:

0.5 µg/mL: Transfer 1.25 mL of the 10-µg/mL standard solution of each analyte to a 25-mL volumetric flask. Bring to volume with ethyl acetate and mix well. Store tightly capped at 1-8°C.

0.05 µg/mL: Transfer 125 µL of the 10-µg/mL standard solution of each analyte to a 25-mL volumetric flask. Bring to volume with ethyl acetate and mix well. Store tightly capped at 1-8°C.

These solutions serve as the source for preparation of all actual GC standard solutions.

Prepare the following concentrations of either separate or combined parent and DPX-JT333 standard solutions, as applicable, in control soil extract:

0.1 µg/mL: Transfer 40 µL of 0.5-µg/mL analyte standard solution to a 400-µL glass insert contained within a glass autosampler vial. Evaporate solvent under a stream of nitrogen at room temperature. Add 200 µL of control sample extract from Step 4.3.4, cap the vial, and mix by vortex.

0.05 µg/mL: Transfer 20 µL of 0.5-µg/mL analyte standard solution to a 400-µL glass insert contained within a glass autosampler vial. Evaporate solvent under a stream of nitrogen at room temperature. Add 200 µL of control sample extract from Step 4.3.4, cap the vial, and mix by vortex.

0.025 µg/mL: Transfer 10 µL of 0.5-µg/mL analyte standard solution to a 400-µL glass insert contained within a glass autosampler vial. Evaporate solvent under a stream of nitrogen at room temperature. Add 200 µL of control sample extract from Step 4.3.4, cap the vial, and mix by vortex.

0.006 µg/mL: Transfer 24 µL of 0.05-µg/mL analyte standard solution to a 400-µL glass insert contained within a glass autosampler vial. Evaporate solvent under a stream of nitrogen at room temperature. Add 200 µL of control sample extract from Step 4.3.4, cap the vial, and mix by vortex.

Store all GC standard solutions between 1°C and 8°C when not in use.

4.2.6 *Source (and Characterization) of Samples*

Since the method requires the use of control sample extract for preparing calibration standards, soil samples should be collected from both treated and untreated locations.

Samples used to validate this method were collected from two DuPont Agricultural Products sites in the United States (Bradenton, Florida, and Madera, California) during a dissipation study (AMR 3402-95) and from test site(s) in Canada, also during a dissipation study (CAN-96-092). Samples were shipped frozen to Morse Laboratories, Inc., in Sacramento, California, where analysis was performed. Soil from the following soil segments were tested: 0 to 15 cm, and 30 to 45 cm.

4.2.7 *Storage and Preparation of Samples*

Soil samples are stored frozen (-10°C or less) until analysis. Before chemical analysis, each sample should be homogenized in a mixer, and stones (i.e., greater than approximately 5-mm diameter) and debris (e.g., wood, plant material, etc.) should be removed.

4.2.8 *Sample Fortification Procedure*

Once the sample is weighed into the extraction vessel (bottle), an appropriate volume (≤1.0 mL) of fortification standard solution should be added to the soil surface. Allow the solvent to evaporate for approximately 5 min before proceeding.

4.2.9 *Analyte Extraction Procedure*

1. Weigh 10.0 g of soil sample into a 250-mL polyethylene centrifuge bottle. **Fortify appropriate samples at this time** (see Section 4.2.8).
2. Add 20.0 mL of extraction solution (10% water/90% acetonitrile) and cap the bottle.
3. Shake the sample on a wrist action shaker for 30 min.
4. Centrifuge for 15 min. at approximately 2400 rpm.
5. Carefully decant the supernatant into a 125-mL, silanized, separatory funnel.
6. Repeat Steps 2, 3, and 4, making sure the soil pellet formed at Step 4 is broken up prior to extraction.
7. Carefully decant the supernatant into the 125-mL separatory funnel containing the supernatant from the first extraction.
8. Add 3.0 g of sodium chloride to the combined extract in the separatory funnel and gently shake for approximately 30 sec. This step forces a separation of the water from the ACN. Allow the layers to separate (approximately 30 min.). Using a glass stirring rod, gently mix the upper acetonitrile layer to insure homogeneity.

9. Transfer a 3.6-mL aliquot of the acetonitrile layer to a silanized, 50-mL centrifuge tube. Label appropriately.

THE ANALYSIS CAN BE STOPPED AT THIS POINT. CAPPED EXTRACTS SHOULD BE STORED FROZEN (-8°C OR LOWER).

4.2.10 *Analyte Purification Procedure*

Check or calibrate the SPE cartridges prior to use in order to ensure optimum method performance. In general, check one cartridge per lot number. This assessment should be conducted well in advance of needing the columns for sample analysis. Analyte recovery greater than 90% indicates that a box of cartridges is suitable for use. Assessment analyses are conducted on a reagent blank (no soil). See Appendix 2 for detailed instructions on assessment of SPE cartridge.

4.2.10.1 *Hexane Washing of ACN extract aliquots*

1. Add 3.6 mL of hexane to each centrifuge tube containing the 3.6 mL of acetonitrile extract, cap and shake the tube for approximately 1 min. Remove and discard the upper hexane layer using a long-stemmed Pasteur pipette.
2. Repeat Step 1.
3. Evaporate the ACN remaining in each tube to near dryness using an N-Evap at 50°C. Gently blow dry with nitrogen at room temperature. Proceed to the next section (Section 4.2.10.2).

4.2.10.2 *Parent & DPX-JT333 Analysis*

Note: SPE cartridges are operated by attaching them to the Vac-Elut apparatus, setting the vacuum so that the effluent forms a steady flow of distinct droplets.

1. Add 2.0 mL of water to the tube containing the evaporated ACN extract (Step 3 of Section 4.2.10.1).
2. Vortex mix each sample (in 2.0-mL water) for approximately 30 sec.
3. Add 10 mL of hexane and mix thoroughly (hand shake) for approximately 30 sec.
4. Draw off the upper hexane layer with a long-stemmed Pasteur pipette and place in another silanized, 50-mL centrifuge tube.
5. Repeat Steps 3 and 4 placing the hexane layer into the 50-mL centrifuge tube containing the hexane layer from the first extraction.
6. Concentrate the combined hexane extracts to approximately 4.0 mL using the N-Evap (water bath set at 40°C).
7. Condition a 5-gram Silica Bond Elut® cartridge by passing 40 mL of hexane through the cartridge. Do not let the cartridge go to dryness after conditioning. (Stop elution when hexane reaches top of frit.) Discard conditioning solvent.

8. After conditioning, pass the sample extract from step 6 through the silica cartridge. Wash the centrifuge tube with two, 1-mL rinses of hexane, passing these through the cartridge as well. Do not let the cartridge go to dryness. Discard eluate.
9. Wash the cartridge with 10 mL of hexane. Discard this wash.
10. Wash the cartridge with 30 mL of 2% isopropanol/98% hexane. Discard this wash.
11. Add 35 mL of 5% isopropanol/95% hexane elution solvent. Discard the first 15 mL of this solvent.
12. Collect the remaining eluate in a silanized 16 × 150-mm test tube and reduce the volume to approximately 0.5 mL on the N-Evap (water bath set at 40°C).
13. Quantitatively transfer the sample to a silanized 13 × 100-mm test tube with small amounts of ethyl acetate and continue the evaporation using the N-Evap at 40°C to approximately 0.2 mL.
14. Evaporate the organic solvent with a stream of nitrogen at room temperature, and re-dissolve the sample residue in 1.0 mL of ethyl acetate. This solution represents 1.0 g of soil sample. Submit to GC analysis.

4.2.11 *Derivatization Procedure*

No derivatization is necessary in this method.

4.3 Instrumentation

4.3.1 *Description*

The method was developed and validated on a Hewlett-Packard model HP5890E gas chromatograph equipped with a HP5972 mass selective detector and a HP7673 autosampler, and an HP G1034C MS ChemStation. Instrument conditions follow.

4.3.2 *Instrument Operating Conditions*

Column:	10-m × 0.20-mm i.d. fused silica column crossbonded with a 0.33-µm film of DB-1
Inlet Liner:	2 mm i.d. gooseneck splitless liner lightly packed with fused silica wool
Injection Volume:	4 µL
Carrier Gas:	helium
Column Head Pressure:	12.8 psi
Purge Flow Timing:	on at 1.00 min.
Temperatures:	Injector: 300 °C GC/MSD transfer line: 295°C Column: Initial: 240°C Rate: 20°C/min.

	Final: 295°C, hold for 2.75 min	
Tuning:	Prior to analysis, the instrument is tuned manually for ion m/e 502.	
Ions Monitored:	Parent m/e 527	Enter this ion 3 times into Group 1 of the SIM acquisition table.
	DPX-JT333 m/e 469	Enter this ion 2 times into Group 2 of the SIM acquisition table. This arrangement will improve sensitivity for the instrument.
Dwell Time:	50 msec	
Retention Times:	approximately 3.0 minutes for Parent approximately 3.5 minutes for DPX-JT333	

4.3.3 *Calibration Procedures*

Prepare a four-point standard curve by injecting analyte standard solutions prepared in control-sample extract. Preparation of additional control-sample extract in each analytical set will be necessary to prepare GC standard. Guidance on the amount of control-sample extract is provided in the following section. All injections (analyses) of standard solutions are used to construct the standard curve.

4.3.4 *Sample Analysis*

This method relies on the preparation of calibration samples in control soil extract to offset matrix enhancement. Matrix enhancement is a phenomenon often observed in GC-MS analytical methods in which the signal of analytes in matrix extract is considerably higher than in pure solvents. The outcome of matrix enhancement is that recovery of analyte in fortified samples is consistently very high (e.g., 150% or higher). Several attempts (dilution, clean-up, injection additives, etc.) were made to eliminate enhancement, but were unsuccessful. Therefore, control matrix extract is required to "control" the enhancement phenomenon.

Inject a curve check standard after analyzing every 4 or 5 samples.

If the final-sample extract requires dilution for the response to be within the range of standard curve responses, then the final-sample extract should be diluted with control-sample extract and re-injected. The table below describes a typical analytical set and the corresponding amount of control-sample extract required. Experience with parent and DPX-JT333 analysis in soil has shown that final extracts of fortification samples typically require one dilution, while those of field samples may require up to three dilutions. It is unlikely that surveillance sample extracts would require dilution.

Sample Set Description	Volume of control sample extract required
GC standard solutions: (4 solutions × 200 µL/solution)200 µL/solution)	800 µL
1 control sample	0 µL
1 fortification requiring no dilution	0 µL
1 fortification requiring dilution	200 µL
8 samples, 6 of which are likely to require dilution (6 samples × 3 dilutions/sample × 200-µL/dilution)	3600 µL
Total	**4600 µL**

Starting at Step 9 of Section 4.2.9, process five additional 3.6-mL aliquots of control extract through the remainder of the procedure. This will yield five 1-mL portions (a total of 5 mL) of control sample extract. Combine all extracts generated into a single solution prior to use.

4.4 Calculations

4.4.1 Methods

Calculation of residue concentrations from instrumental data were conducted using a validated software application. Standard least-squares regression was used to calculate a best-fit line based on known concentrations (standards solutions) and the resultant peak area from the analysis of those solutions. The line was then used to determine concentrations of the analyte found during sample analysis.

The equation used for the calibration line was

$$y = mx + b$$

where: y = net peak area response (response in fortification *less* response in control),
x = µg analyte/mL,
m = slope of the regression line, and
b = y-intercept.

Therefore, the µg analyte/mL value for each sample was calculated according to the following adaptation of the regression equation:

$$x = (y - b) / m$$

The concentration (ppm) of each analyte in a residue sample was calculated according to the following equation.

$$\text{ppm of analyte} = \frac{\mu\text{g analyte / mL} \times \text{mL solvent} \times \text{mL of final volume} \times \text{GC dilution factor}}{\text{g of sample} \times \text{mL of aliquot}}$$

where,

µg analyte/mL	was derived in the regression line from the area of the analyte peak,
mL solvent	was the volume of ACN portion of the combined extraction solvent,
mL of aliquot	was the volume of ACN extract taken through cleanup steps,
g of sample	was the weight of the portion of sample extracted,
mL of final volume	was the volume of final extract submitted to analysis, and
GC dilution factor	was the magnitude of dilution required to bracket the response of the sample within the standard curve responses. When the sample requires no dilution, the GC dilution factor equals 1.

The percent recovery for fortified control samples was calculated as follows:

$$\%\text{Recovery} = \frac{(\text{ppm of analyte measured in fortified sample} - \text{ppm measured in control sample}) \times 100\%}{\text{ppm of analyte added to sample}}$$

4.4.2 *Examples*

The following example calculations were for a DPX-KN128/IN-KN127 fortification at 1.0 ppm (Spike 43 of Set #16; Figure 7) extracted 17 December 1996 and analyzed 19 December 1996. Please refer to Table 2 for the results of the ppm-of-analyte and percent-recovery calculations.

µg analyte/mL = ((113,833 area counts - 0 area counts) - (-3164 area counts) ÷ (2,537,000 area counts x mL/µg)

= 116,997 area counts ÷ (2,537,000 area counts x mL/µg)

= 0.0461 µg/mL

ppm of analyte = (0.0461 µg/mL x 36 mL x 1.0 mL x 20) ÷ (10.0 g x 3.6 mL)

= (33.19 µg x mL) ÷ (36 g x mL)

= 0.922 µg/g

= 0.92 ppm

% Recovery = ((0.92 ppm - 0 ppm) x 100%) ÷ 1.0 ppm

= 92%

5.0 RESULTS AND DISCUSSION

5.1 Method Validation Results

Data for this section were collected in validation Set 16 for a field dissipation study being conducted in Canada (DuPont Canada Study No. CAN 96-902).

5.1.1 Detector Response

Regression statistics (Table 1, Figures 1 and 2) for the two analytes indicated excellent correlation between concentration and detector response. The test compound is noted on the figures as DPX-KN128/KN127, which recalls that the two stereoisomers are not resolved in the GC analysis and that they co-exist in the standard material. Correlation coefficients (r-statistic) ranged from 0.9993 to 0.9997, while y-intercepts (b-statistic for linear regression) were very close to the origin (zero), relative to the average response.

Peaks for calibration standards (in matrix extract) for parent and DPX-JT333 (Figures 3 and 4) indicated retention times of approximately 3.05 min and 3.45 min, respectively. The peaks at the lowest concentration (0.006 ppm) were clearly quantifiable (i.e., approximately 10- to 20-times background). No matrix peaks were observable in the baseline in the response within 1 min of either analyte peak.

5.1.2 Controls

The background response in the analysis of parent & DPX-JT333 (Figure 5) was essentially flat. That is, there were no apparent interferences from the matrix in the region where either analyte emerged.

5.1.3 Recoveries (Accuracy and Precision)

Method development and validation were performed at Morse Laboratories, Inc. Three fortification concentrations (0.010 ppm, 1.0 ppm, and 10 ppm) were analyzed in triplicate for each of two combined soil segment samples: 0 to 15 cm and 30 to 45 cm (Table 2). Six analytical sets were extracted over a period from 18 September to 17 December 1996, with each set being comprised of all fortification concentrations for a single soil segment. Therefore, extractions were conducted on

three dates, and each replicate for each concentration and segment was therefore an independent assay.

At LOQ (0.010 ppm), average recoveries for both analytes and both segment depths ranged from 97% to 107%. Average recoveries were typically lower for 1.0-ppm fortifications (88% to 100%) and 10-ppm fortifications (84% to 101%). All recovery results were within the acceptable range of 70 to 120% of nominal, indicating the method performed satisfactorily.

5.1.4 *Extraction Efficiency*

Extraction efficiency was tested in an aerobic soil metabolism study (DuPont Study No. AMR 2803-93). The soil used in that study was a silt loam (organic matter = ~2.5%). A series of four solvents, ranging from polar to non-polar, were used in the test. Essentially all of the extractable residue was removed in the first solvent in the series (acetonitrile:water, 9:1, v:v). The data clearly indicated that weathering the soil reduced the amount of extractable residue from approximately 100% the day of treatment to approximately 25% 1 year later. The method was found to be efficient in removing available (non-bound) residues, even from weathered soils.

5.1.5 *Limit of Quantitation (and LOD, if one is to be specified)*

5.1.5.a *Background Evaluation*

In a sample fortified at 0.010 ppm (Figure 6), background noise in the area of parent and DPX-JT333 was measured (using a metric ruler) to be approximately 0.5 mm, while the parent peak was measured at 6 mm. These measurements gave a signal-to-noise (S-N) ratio of 12 to 1. The S-N ratio for the lowest standard (0.006 µg/mL), which was selected to be approximately 70% of the expected LOQ response, was measured to be 8 to 1 (Figure 3). These data indicated that the LOQ met existing DuPont guidance for this parameter (S-N of 5 to 20).

5.1.5.b *Limits Determination/Calculation*

The LOQ was determined by background assessment (Section 5.1.5.a) and through assessing the recovery at 0.01 ppm of each analyte. The background assessment indicated that the S-N ratio for the LOQ fortification (0.010 ppm) was within DuPont guidance. Also, recoveries at LOQ were between 70% and 120% at least 80% of the time. Therefore, given the current method procedures, the LOQ is properly set. The limit of detection (LOD) is therefore taken to be one-third of the LOQ (0.003 ppm).

5.2 ***Timing***

Approximately 10 hours are needed to prepare each set for analysis. Note that a set was comprised of 8 samples: one reagent blank, one soil control, 2 soil fortifications (one at LOQ and the other at a higher level), and 2 to 4 field samples. Instrumental analysis requires approximately 2 hours for the samples and the attendant calibration standards, which can be run in an unattended fashion with an autosampler. Data

analysis and reduction require approximately 1 hour per set. Therefore, the analysis can be completed in 12 to 14 hours.

5.3 Modifications or Special Precautions

It is imperative that a clean separation of phases be produced in the raw extract solvent (ACN:water). If this is not achieved, subsequent steps in the clean-up and analysis may be seriously compromised.

5.4 Method Ruggedness

5.4.1 Stability

The method appears to perform consistently over a long duration. The method has been successfully used for data generation purposes in a field dissipation program conducted in the U.S. since 1995 (DuPont Study Nos. AMR 3294-95 and AMR 3402-95) and is being used in a dissipation study in Canada (DuPont Study No. CAN-96-902.

5.4.2 Specificity/Potential Interference

5.4.2.a Interference from glassware and reagents, matrices

The method is a single-ion monitoring (SIM) method employing mass-spectral detection. Therefore, it has considerable inherent specificity. However, this method, as are other methods employing such detection, is subject to matrix enhancement phenomenon; hence, the use of standards in soil extract. A closely related soil (adjacent to treated field) could be used as a control in this assay. Since the test substance does seem to remain located in the upper 1 cm (approx.) of the soil surface, a control could be collected by carefully removing that upper surface and taking soil in the 5- to 15-cm horizon.

5.4.2.b Interference from other pesticides

This method was not tested for interference by other pesticides.

5.4.3 Confirmatory Method

This method has inherent confirmation of structure due to the detection by mass spectrometry. Additional ions or an entire mass spectrum could be monitored for structural identification. No confirmatory analysis by another technique should be necessary.

5.4.4 Second Lab Tryout

This method has not been tested in a second lab due to resource availability and timing issues.

6.0 CONCLUSIONS

The subject method was developed for measuring DPX-KN128 residues (measured as DPX-KN128/IN-KN127 and DPX-JT333) in soil samples. Soil is extracted with ACN:H_2O and analytes are measured using GC-MSD operated under SIM conditions. Results from the analytes are related back to DPX-KN128/IN-KN127 concentrations for reporting purposes. The method has been used successfully to generate residue data from three soils in Canada and two soils in the U.S. The LOQ of the method is 0.01 ppm of parent equivalents from either DPX-KN128/IN-KN127 (parent) or a known metabolite, DPX-JT333.

7.0 RETENTION OF RECORDS

Data for this revision and the final report are retained in the GLP Archives located at:

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

Laboratory-specific or site-specific raw data such as personnel files, instrument, equipment, refrigerator, and/or freezer raw data will be retained at the facility where the work was done.

8.0 REFERENCE

1. Westberg, G.L., 1997. *Determination of DPX-JW062 and Its Metabolites DPX-JT333 and IN-KG433 in Soil*, Morse Laboratories, Inc., SOP# Meth-91, Revision #5.

TABLES

27

TABLE 1
LINEARITY OF DETECTOR RESPONSES

ANALYTE	SOIL	PPM	NO. STDS.	STATISTICS
Parent (DPX-KN128/	Canada-C	0.006	2	r = 0.9997
IN-KN127)	Core 0-15 cm	0.025	1	m = 25,337,000
		0.050	3	b = -3164
		0.10	1	
DPX-JT333	Canada-C	0.006	2	r = 0.9993
	Core 0-15 cm	0.025	1	m = 2,436,000
		0.050	3	b = -3540
		0.10	1	

TABLE 2
RESULTS OF ANALYSIS FOR DPX-KN128 AND DPX-JT333 IN SOIL

ANALYTE	PPM FORTIFIED	CM DEPTH	NO. OF ANALYSES	% RECOVERY
Parent: DPX-KN128/IN-KN127	0.010	0-15	3	110, 110, 100 Avg. = 107 ± 5.8
		30-45	3	110, 100, 100 Avg. = 103 ± 5.8
	1.0	0-15	3	92, 100, 85 Avg. = 92 ± 7.5
		30-45	3	98, 110, 92 Avg. = 100 ± 9.2
	10	0-15	3	86, 96, 120 Avg. = 101 ± 17
		30-45	3	98, 83, 86 Avg. = 89 ± 7.9
DPX-JT333	0.010	0-15	3	110, 100, 82 Avg. = 97 ± 14
		30-45	3	100, 95, 97 Avg. = 97 ± 2.5
	1.0	0-15	3	89, 100, 75 Avg. = 88 ± 13
		30-45	3	99, 110, 92 Avg. = 100 ± 9.1
	10	0-15	3	82, 89, 110 Avg. = 94 ± 15
		30-45	3	91, 80, 81 Avg. = 84 ± 6.1

FIGURES

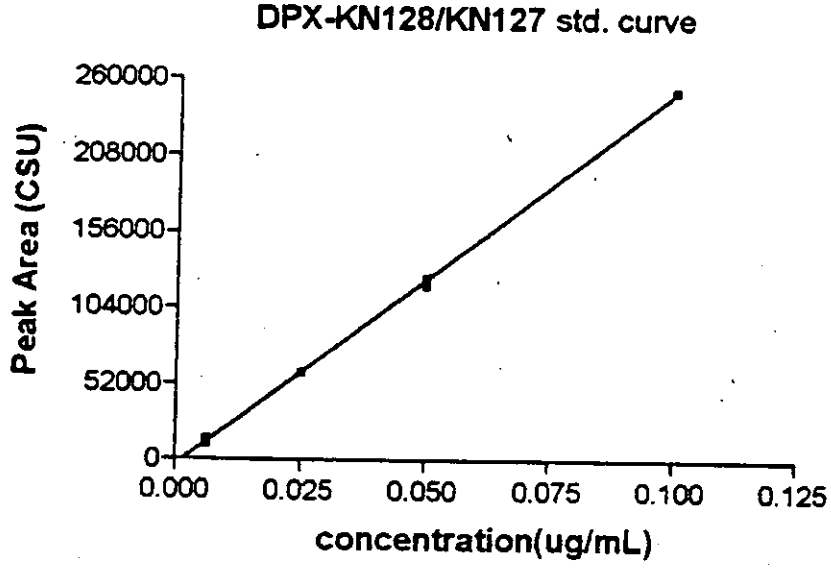
FIGURE 1
DPX-KN128/IN-KN127 STANDARD CURVE

Pg 1 of 3

Conc(ug/mL)	Pk.Area
0.100	252048
0.006	11582
0.025	59961
0.050	120088
0.050	123183
0.050	124578
0.006	14448

ML96-0643-DUP
Protocol No: CAN 96-902
Set #16-Soil (DPX-KN128/KN127)
Method Validation

December 19,1996



- Pk.Area in Chemstation units

r= 0.9997
m= 2537000 ± 26340
b= -3164 ± 1343

Equation:y=mx+b

12/30/96

31

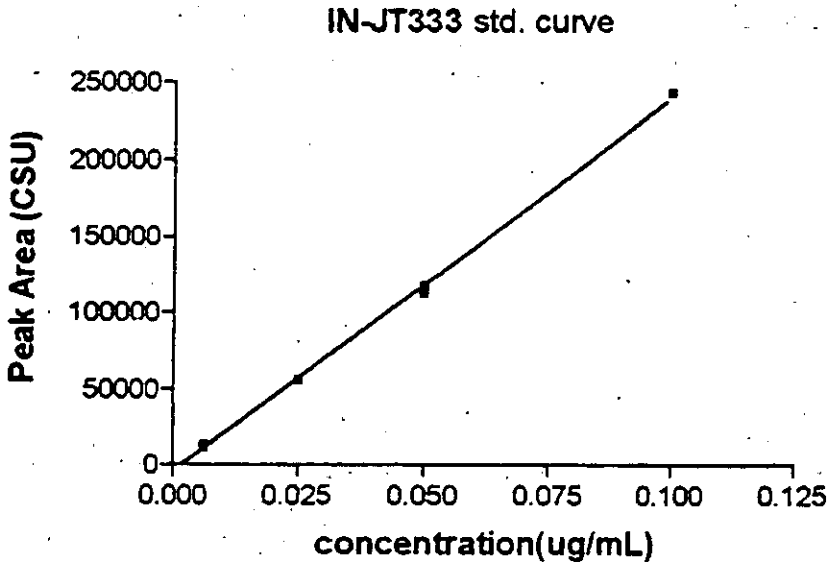
FIGURE 2
DPX-JT333 STANDARD CURVE

Pg 1 of 3

Conc(ug/mL)	Pk.Area
0.100	243519
0.006	12768
0.025	56621
0.050	113364
0.050	118024
0.050	116218
0.006	13759

ML96-0643-DUP
Protocol No: CAN 96-902
Set #16-Soil (IN-JT333)
Method Validation

December 19,1996



- Pk.Area in Chemstation units

r= 0.9993
m= 2436000 ± 39740
b= -3540 ± 2026

Equation:y=mx+b

12/30/96

32

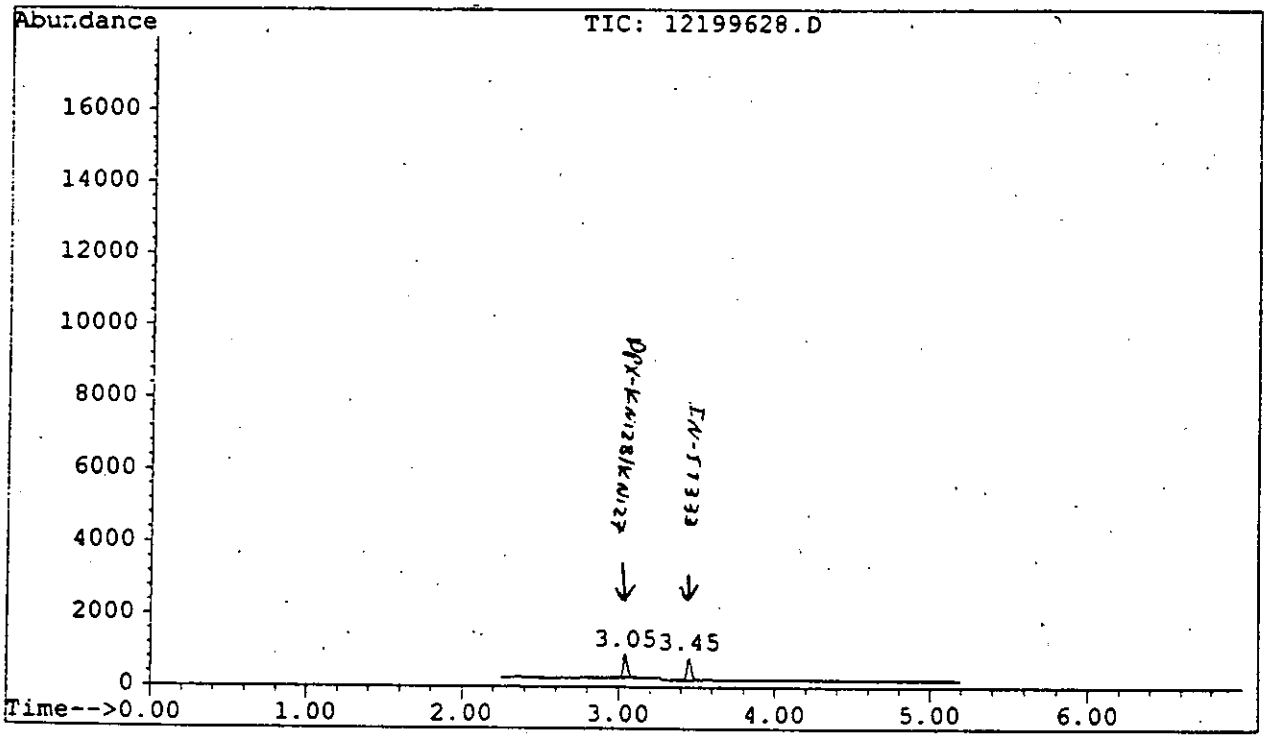
FIGURE 3
STD: 0.006-µG/ML DPX-KN128/IN-KN127 AND IN-JT333 IN CONTROL MATRIX EXTRACT

2+1L

Area Percent Report -- Sorted by Retention Time

Information from Data File:
File : C:\HPCHEM\1\DATA\12199628.D
Operator : KLC
Acquired : 19 Dec 96 10:08 pm using AcqMethod MP062
Sample Name: DPX-KN128/KN127 0.006 ug/ml 4 ul IN-JT333 @ 11-25-96
Misc Info : (DC-UTC2.2(b) control matrix)
Vial Number: 11
CurrentMeth: C:\HPCHEM\1\METHODS\MP062.M

CURVE STANDARD
ID 12/19/96 Sct #16



Ret Time	Signal Descr	Area	% Pk	%LPk
3.046	TIC	11582	100.000	100.000
3.454	TIC	12768	100.000	100.000

C:\HPCHEM\1\DATA\12199628.D Thu Dec 19 22:14:34 1996

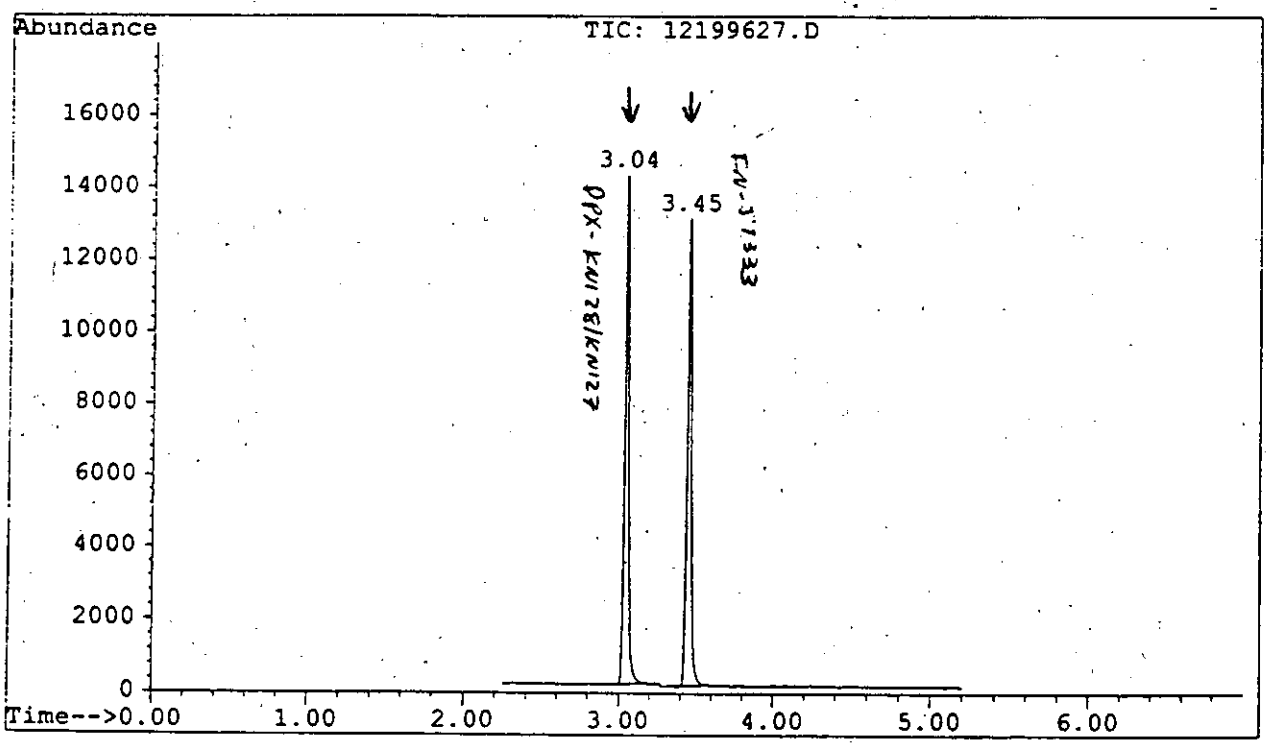
FIGURE 4
STD: 0.10-µG/ML DPX-KN128/IN-KN127 AND IN-JT333 IN CONTROL MATRIX EXTRACT

1 of 2

Area Percent Report -- Sorted by Retention Time

Information from Data File:
File : C:\HPCHEM\1\DATA\12199627.D
Operator : KLC 12-23-96
Acquired : 19 Dec 96 9:59 pm using AcqMethod MP062
Sample Name: DPX-KN128/KN127 0.10 ug/ml 4 ul IN-JT333 @ 12-23-96
Misc Info : (DC-UTC2.2(b) control matrix)
Vial Number: 14
CurrentMeth: C:\HPCHEM\1\METHODS\MP062.M

CURVE STANDARD
ID 12/19/96 Set #16



Ret Time	Signal Descr	Area	% Pk	%LPk
3.040	TIC	252048	100.000	100.000
3.448	TIC	243519	100.000	100.000

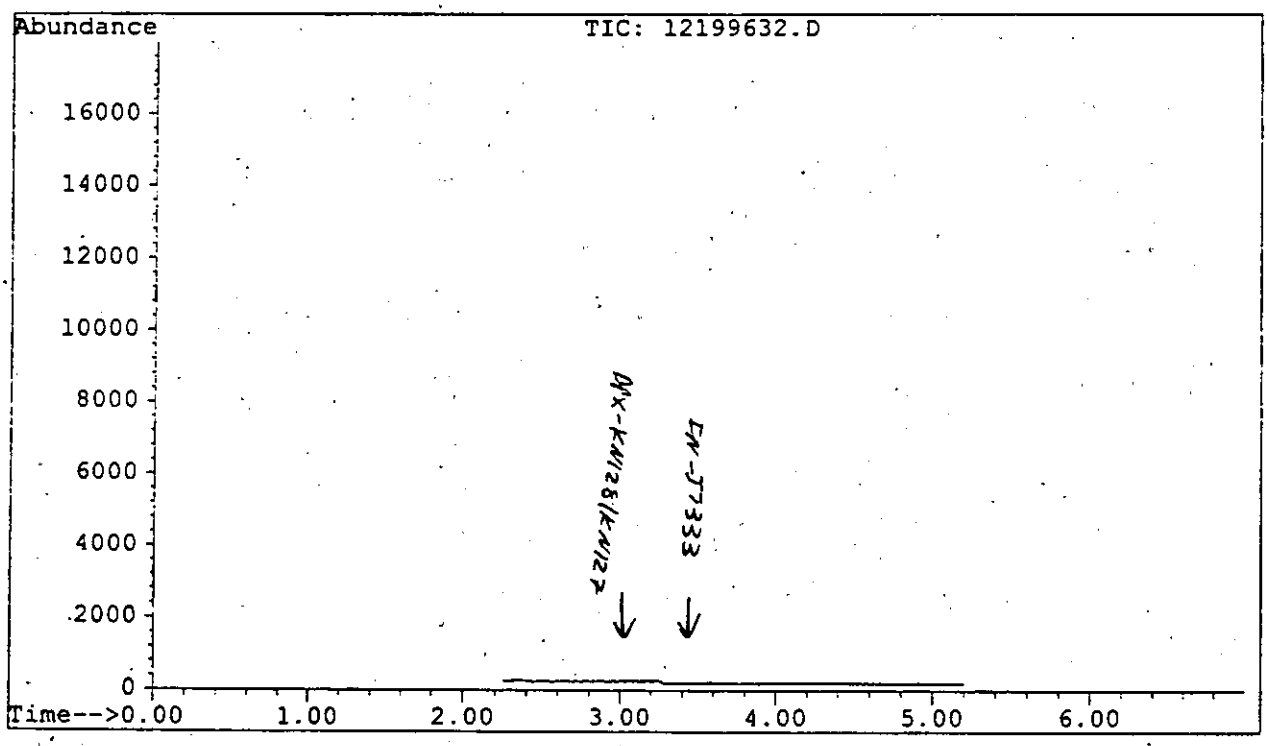
C:\HPCHEM\1\DATA\12199627.D Thu Dec 19 22:06:10 1996

FIGURE 5
STD: 0-µG/ML DPX-KN128/IN-KN127 AND IN-JT333 IN CONTROL MATRIX EXTRACT

6 of 12

Area Percent Report -- Sorted by Retention Time

Information from Data File:
File : C:\HPCHEM\1\DATA\12199632.D
Operator : KLC
Acquired : 19 Dec 96 10:41 pm using AcqMethod MP062
Sample Name: Set #16 73106 DPX-KN128/KN127 Soil MV IN-JT333 KLC 12-22-96
Misc Info : DC-UTC2.2(b) , Check 19 , 1.0 g/ml , 4 ul
Vial Number: 16
CurrentMeth: C:\HPCHEM\1\METHODS\MP062.M



Ret Time	Signal Descr	Area	% Pk	%LPk

C:\HPCHEM\1\DATA\12199632.D Thu Dec 19 22:48:13 1996

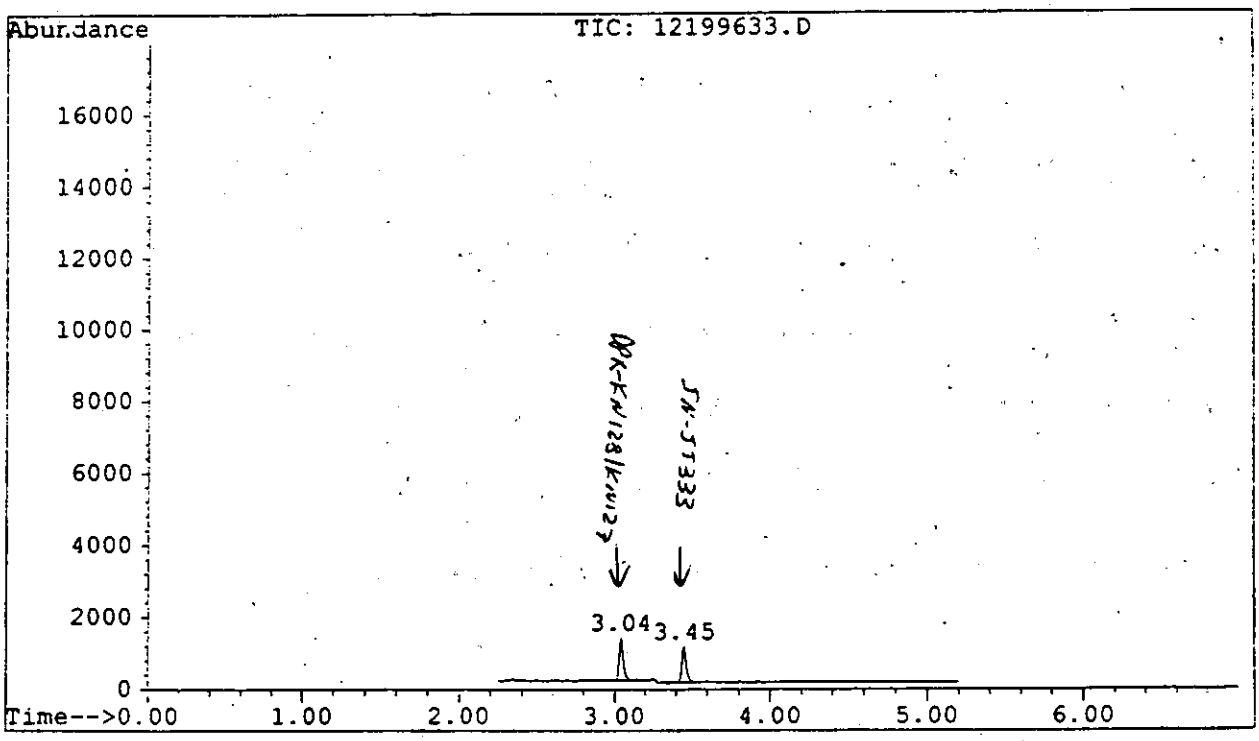
35

FIGURE 6
STD: 0.010-PPM DPX-KN128/IN-KN127 AND DPX-JT333 FORTIFICATION

7 of 12

Area Percent Report -- Sorted by Retention Time

Information from Data File:
File : C:\HPCHEM\1\DATA\12199633.D
Operator : KLC
Acquired : 19 Dec 96 10:50 pm using AcqMethod MP062
Sample Name: Set #16 73106 DPX-KN128/KN127 Soil MV IN-JT333 (10) BLL 12-23-96
Misc Info : DC-UTC2.2(b),Spk.42 0.010ppm,1.0 g/ml, 4 ul
Vial Number: 17
CurrentMeth: C:\HPCHEM\1\METHODS\MP062.M



Ret Time	Signal Descr	Area	% Pk	%LPk
3.045	TIC	22525	100.000	100.000
3.455	TIC	20139	100.000	100.000

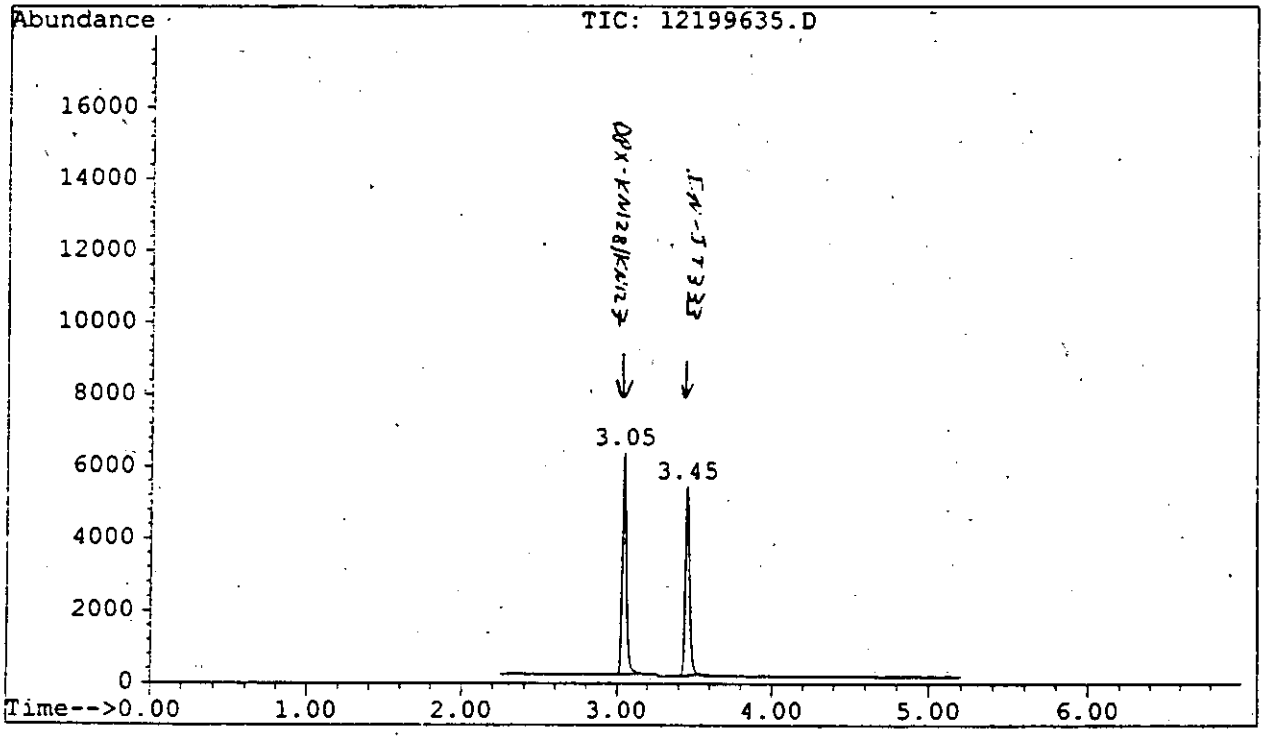
C:\HPCHEM\1\DATA\12199633.D Thu Dec 19 22:56:38 1996

FIGURE 7
STD: 1.0-PPM DPX-KN128/IN-KN127 AND DPX-JT333 FORTIFICATION

9+12

Area Percent Report -- Sorted by Retention Time

Information from Data File:
File : C:\HPCHEM\1\DATA\12199635.D
Operator : KLC
Acquired : 19 Dec 96 11:07 pm using AcqMethod MP062
Sample Name: Set #16 73106 DPX-KN128/KN127 Soil MV IN-JT333 (a) KLC 12-23-96
Misc Info : DC-UTC2.2(b),Spk.43 1.0ppm,1.0g/ml,4ul,1-20dil (b) KLC 12-23-96
Vial Number: 18
CurrentMeth: C:\HPCHEM\1\METHODS\MP062.M



Ret Time	Signal Descr	Area	% Pk	%LPk
3.046	TIC	113833	100.000	100.000
3.454	TIC	101338	100.000	100.000

C:\HPCHEM\1\DATA\12199635.D Thu Dec 19 23:13:26 1996

APPENDICES

APPENDIX 1
SILYLATION OF GLASSWARE

Silylation of Glassware

Purpose:
Silylation is a process used to chemically treat glassware in order to prevent or minimize binding of analyte residues to the glass surface.

Safety Considerations:
This procedure must be done in a fume hood. The person performing this procedure must wear heavy latex gloves. The silylating reagent, dimethyldichlorosilane, must NOT come in contact with water, otherwise chlorine gas and hydrogen chloride gas, both severely toxic, will be produced.

Procedure:

1. Prepare 100 mL of a 5% (v/v) solution of dimethyldichlorosilane (DMDCS) in hexane.

   Add hexane (95 mL) to a glass stoppered glass container (approximately 200 mL volume). Slowly add 5 mL of DMDCS. Stopper the container and invert to mix.

   Larger volumes may be prepared using the proportions discussed above. However, it is prudent to prepare amounts that will be entirely used in order to avoid storage and disposal of excess solution.

2. Pour a small amount of the DMDCS solution into the glassware to be treated. Rotate the glassware to thoroughly coat the inside surfaces. Pour excess solution into the next piece of glassware to be treated.

3. Allow the treated glassware to dry (approximately 20 minutes). Rinse with de-ionized water, then acetone. Again allow to dry.

4. Glassware is now ready for use.

Notes:
- Any glassware that is cleaned with a brush after it has been silylanized, must be re-treated.
- Store pure DMDCS at room temperature.
- 5% solutions of DMDCS in hexane are stable for 5 days when stored well-stoppered at room temperature.

APPENDIX 2
QUALITY CONTROL PROCEDURE FOR SILICA SPE CARTRIDGES

Quality Control for SPE Cartridges

Silica Cartridges

DPX-KN128/IN-KN127 & DPX-JT333
Add 100 µL of 10 µg/mL combined DPX-JW062 and DPX-JT333 standard (in ethyl acetate) to a 50-mL silanized centrifuge tube. Gently evaporate to dryness with nitrogen, and add 4 mL of hexane. Follow Steps 7 through 14 of Section 4.2.10.2 in procedure. Final concentration should be 1.0 µg/mL for each analyte.

IN-KG433
Add 100 µL of 10 µg/mL IN-KG433 standard (in ethyl acetate) to a 50-mL silanized centrifuge tube. Gently evaporate to dryness with nitrogen and add 4 mL of 5% ethyl acetate/95% toluene. Follow Steps 2 through 8 of Section 4.2.10.3 in procedure. Final concentration is 2.0 µg/mL.