Analytical Method for the Determination of KIH-2031 (DPX-PE350) in Water Using Column-Switching Liquid Chromatography

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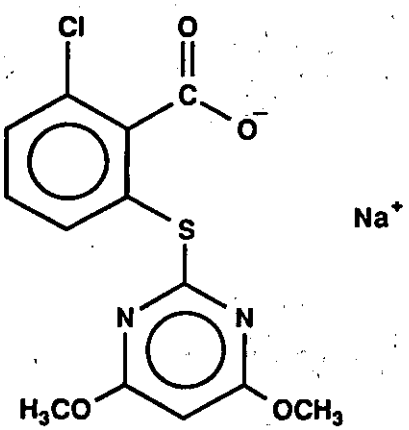
Abstract

An analytical method was developed and is described for the determination of KIH-2031 (sodium 2-chloro-6-[(4,6-dimethoxypyrimidin-2-yl)thio]benzoate, DPX-PE350) extracted from water. DPX-PE350 is extracted from 200 mL of water using a graphitized carbon, solid-phase extraction cartridge. The instrumental method is based on the use of column-switching HPLC with UV absorbance detection at 254 nm. The method detection limit for DPX-PE350 in water is 0.051 ppb (0.051 ng/mL).

The chromatographic separation requires one high-pressure switching valve which places two analytical columns in series. The first column is used as a "clean-up" column from which DPX-PE350 is transferred to the second column. The transfer begins just before the mean elution time of DPX-PE350 standards. After transfer of the analyte to the second column, the valve is switched to allow flow through the first column only to allow elution (clean-up) of other matrix components. Following clean-up, the valve is switched to allow flow to pass through the both columns and the analytical separation of DPX-PE350 is performed.

INTRODUCTION

This analytical method was developed to satisfy U.S. EPA registration requirements for Staple® Herbicide. This method determines residues of DPX-PE350 extracted from water. Staple® Herbicide is used to control broadleaf weeds in cotton. The formulated product contains 85% by weight KIH-2031 (sodium 2-chloro-6-[(4,6-dimethoxypyrimidin-2-yl)thio]benzoate, DPX-PE350). KIH-2031 is referred to as DPX-PE350 in this report. The chemical structure for DPX-PE350 is shown below:



DPX-PE350

Bates (see Reference 1) has determined the following physico-chemical properties for DPX-PE350:

Melting Point:	233.8-234.2°C
Solubility:	
Water	728 g/L
Methanol	270 g/L
Acetone	812 mg/L
Acetonitrile:	347 mg/L
Partition Coefficient, n-octanol/pH 7 water:	0.14
Dissociation Constant, pK_a	2.34

DPX-PE350 was extracted from 200 mL of fortified water by passing the water through a graphitized carbon solid-phase extraction cartridge. After rinsing the cartridge with solutions that selectively removed interfering matrix, DPX-PE350 was eluted from the cartridge.

For HPLC analysis, the following procedure was used. A 0.2-mL sample was injected on a Zorbax® SB-CN column. A valve was switched at a window of transfer that was determined from the average elution time of DPX-PE350 standards eluting off the SB-CN column. This procedure transferred DPX-PE350 from Column I to a second column, a Zorbax® SB-C18 column. After DPX-PE350 was transferred to the second column, the valve was switched to allow the mobile phase to flow through the first column only which allowed elution (clean-up) of other matrix components. Following solvent clean-up, the valve is switched to allow mobile phase to flow through both columns. The analytical separation of DPX-PE350 was performed on the second column with both columns in series.

EXPERIMENTAL

Equipment

Equivalent equipment may be substituted unless otherwise indicated.

Liquid Chromatograph

HPLC System, Waters (Millipore, Inc., Milford, Mass.):

- Pump control module, Waters;
- Three pumps, Waters, Model 510; Note: a three pump, high-pressure mixing HPLC system is not required for this method; a single pump, low-pressure mixing HPLC system will work too.
- Photo diode array detector, Waters, Model 996;
- Raytest, Ramona 5LS radioisotope detector with 370-µL flow cell volume, glass scintillator (Raytest USA Inc., Pittsburgh, Penn.)
- Millennium 2010 v2.00 software run on a NEC 486/33 computer, Waters;
- Auto injector, Waters, Model 717 equipped with a 2.5-mL syringe;
- Temperature control module, Waters;
- Column heater module, Waters; and
- Six-port switching valve, (Valco Inst., Houston, Tex., Model E60, #EC6W)

HPLC Columns

Pre-Column 1	DuPont Zorbax® SB-CN 4.0 x 12.5 mm, 5-µ Reliance Cartridge Guard Column, #820674-916 and column end-fittings, #820529-901 (MAC-MOD Analytical Inc., Chadds Ford, Pa.), **do not substitute**.
Column 1	DuPont Zorbax® SB-CN 4.6 x 150 mm, 5-µ analytical column, #883975-905 (MAC-MOD Analytical Inc., Chadds Ford, Pa.), **do not substitute**.
Column 2	DuPont Zorbax® SB-C_{18} 4.6 x 250 mm, 5-µ analytical column, #880975-902 (MAC-MOD Analytical Inc., Chadds Ford, Pa.), **do not substitute**.

Solid-Phase Extraction Apparatus

Solid-phase extraction manifold, #5-7044M, with disposable Teflon® solvent guides, #5-7059 (Supelco, Bellefonte, Pa.).

Solid-Phase Extraction Cartridges and Adapters

6-mL Envi-Carb tubes #5-7094M (Supelco, Bellefonte, Penn.), **do not substitute**. 75-mL reservoir #1213-1030 and adapter #1213-1003 (Varian Sample Preparation Products, San Fernando, Calif.).

Evaporator

N-Evap® Model 111 laboratory sample evaporator/nitrogen manifold fitted with Teflon®-coated needles (Organomation Associates, South Berlin, Mass.). Unit is attached to a dry, clean nitrogen source.

Mobile Phase Filters and Vacuum Filter Apparatus

Use 0.45-µm pore, Cat. No. HATF 047 00, Type HA filters for pH 3, 30 mM potassium phosphate buffer. Use 0.5-µm pore, Cat. No. FHUP 047 00, Type FH filters for acetonitrile. The Millipore vacuum filter apparatus used to filter and degas mobile phases consists of a glass filter holder, #XX1004700, a ground glass base with stopper, # XX1004702, a funnel cover, #XX2504754, and a 1-L filter flask, #XX1004705 (Millipore, Inc., Milford, Mass.).

Sample Water Filters

For sample water that has not been filtered, use Whatman # 3 Filter # 28456-065 (VWR Scientific Co., Bridgeport, N.J.). For water samples that have been extracted and cleaned up, use Millipore SJHVL04NS, 0.45-µm Type HV filters (Millipore, Inc., Milford, Mass.).

Syringes

2.5-mL disposable plastic syringe, Part No. Z11685-8 (Aldrich Chemical Co., Milwaukee, Wis.); Hamilton 100- and 500-µL syringes, #80600 and #80800, respectively (Hamilton, Reno, Nev.).

pH Meter

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

Balances

Mettler A163 analytical balance (Mettler Instrument Corp., Hightstown, N.J.).

Ultrasonic Bath

Branson Model 2200 ultrasonic bath (VWR Scientific Co., Bridgeport, N.J.).

Mixer

Vortex Genie 2 (VWR Scientific Co., Bridgeport, N.J.).

Pipettes

Pipetman #P-1000 adjustable pipette and EDP-Plus pipette #EP-10ML (Rainin, Emeryville, Calif.).

Graduated Cylinders

Kimax 10-, 100-, 250-, 500-, and 1000-mL graduated cylinders, #24713-503, #24713-111, #24713-144, #24713-166, #24713-188, respectively (VWR Scientific Co., Bridgeport, N.J.).

Volumetric Flasks

Pyrex 25-, 100-, 200-, 500-, 1000-mL volumetric flasks, #29619-212, #29619-234. #29619-245, #29619-267, and #29619-278, respectively (VWR Scientific Co., Bridgeport, N.J.).

Water Filtering Apparatus

Porcelain Buchner funnel, 100-mm diameter, #30310-120 (VWR Scientific Co., Bridgeport, N.J.) with a Neoprene No. 7 stopper, #59589-256 (VWR Scientific Co., Bridgeport, N.J.), and a 500-mL filter flask, # 29415-100 (VWR Scientific Co., Bridgeport, N.J.).

Autosampler Vials

Waters 4-mL vials #72710 with low volume glass inserts and springs #72704 (Millipore, Milford, Mass.).

Pyrex Centrifuge Vial

Pyrex, conical, graduated, 15-mL centrifuge vial with stopper, #21048-027 (VWR Scientific Co., Bridgeport, N.J.).

Reagents and Standards

Equivalent reagents may be substituted for those listed below. To determine if substituted reagent impurities interfere with DPX-PE350, appropriate amounts of the solvents should be injected into the HPLC using the chromatographic conditions specified in this report for DPX-PE350.

Water

Deionized water passed through a Milli-Q® UV Plus water purification system #ZD60 115 UV (Millipore, Bedford, Mass.).

Potassium Phosphate, Monobasic, Crystal (KH_2PO_4)

Baker analyzed potassium phosphate, monobasic, crystal reagent, #3246-05 (J. T. Baker, Phillipsburg, N.J.).

Dichloromethane (DCM)

EM Omni Solv®, residue grade dichloromethane, #DX0831-1 (EM Science, Gibbstown, N.J.). ***Warning*** - dichloromethane is a suspected carcinogen. Use in a fume hood.

Methanol (MeOH)

EM Omni Solv®, HPLC-grade methanol, #MX0488-1 (EM Science, Gibbstown, N.J.).

Acetonitrile (ACN)

EM Omni Solv®, HPLC-grade acetonitrile, #AX0142-1 (EM Science, Gibbstown, N.J.).

Acetone

EM Omni Solv®, HPLC-grade acetone, #AX0116-1 (EM Science, Gibbstown, N.J.).

Ammonium Carbonate [$(NH_4)_2CO_3$]

Baker Analyzed Reagent, reagent-grade ammonium carbonate #0642-01 (J. T. Baker, Inc., Phillipsburg, N.J.).

Phosphoric Acid (H_3PO_4)

Baker Analyzed Reagent, reagent-grade, concentrated phosphoric acid, #0260-01 (J. T. Baker, Inc., Phillipsburg, N.J.).

Hydrochloric Acid (HCl)

Reagent-grade 12 M hydrochloric acid, #9535-01 (J. T. Baker, Inc., Phillipsburg, N.J.).

Sodium Azide, Practical

Baker sodium azide, practical, #3V015-05 (J. T. Baker, Inc., Phillipsburg, N.J.). See warning in ***Preparation of Solutions*** section of this report.

Formic Acid

EM Suprapur® formic acid, #11670-1 (EM Science, Gibbstown, N.J.).

Acetic Acid

Baker Analyzed glacial acetic acid, #9524-00 (J. T. Baker, Inc., Phillipsburg, N.J.).

12

DPX-PE350

Reference substance used for HPLC analysis: analytical standard grade DPX-PE350, Lot #4, 98.7% pure (prepared by Kumiai/Ihara Chemical Co. for DuPont Agricultural Products, Global Technology Division, E. I. du Pont de Nemours and Company).

Radioactive DPX-PE350, NEN #2764-067, HOTC #370, 99.0% pure. Specific Activity: 70.210 µCi/mg. Radiolabel location: pyrimidine-2-^{14}C.

Water Samples

This method was developed and validated using water from the following sources:

Wilson Run, New Castle County, Delaware,

Brandywine River, New Castle County, Delaware, and

Groundwater from well in Edgecombe County, North Carolina.

Preparation of Solutions

pH 3, 30 mM Potassium Phosphate Buffer

Add 7.21 g KH_2PO_4, 0.2 g sodium azide (optional, see warning below), and 0.40 mL of 85% H_3PO_4 to 2.0 L of fresh HPLC-grade water in a beaker and dissolve the salt using a magnetic stirrer. Adjust the pH to 3.00 with 85% H_3PO_4 if the pH is higher than pH 3.00 or with 50% KOH if the pH is lower than pH 3.00. This solution should be prepared fresh weekly and should be filtered daily before use with a 0.45-µm pore filter.

Warning - Sodium azide is combustible, shock sensitive, and can explode. Sodium azide is a health hazard and should not be swallowed, inhaled, or absorbed through the skin, and should be disposed of properly. *Potassium phosphate buffers with sodium azide should not be put down the drain.* Read the material safety data sheet for this compound before use. Sodium azide is used in this method as a growth inhibitor for bacteria. It does not need to be used if the phosphate buffer is filtered daily, prepared fresh weekly, and stored in the dark. If sodium azide is not used, the solvent lines from the reservoir to the pump should be purged with acetonitrile weekly to control bacterial growth.

0.01 M Ammonium Carbonate

Dissolve 0.78 g of $(NH_4)_2CO_3$ in about 800-mL distilled water and dilute to 1.00 L in a volumetric flask. This solution should be made weekly.

0.1 M Hydrochloric Acid

Pipet 8.33 mL 12 M HCl into 1-L volumetric flask and bring to volume with Milli-Q® water. This solution should be made weekly.

90% Dichloromethane/10% Methanol

With 1000-mL graduated cylinder, measure 900 mL of dichloromethane and add to 1-L volumetric flask. With 100-mL graduated cylinder, measure 100 mL of methanol and add to the 1-L flask. Do not adjust the volume to 1-L mark. This solution should be made weekly.

0.1 M Formic Acid in 90/10 DCM/MeOH

Pipet 0.755 mL of EM Science Suprapur® formic acid into 200-mL volumetric flask. Bring to volume with 90% DCM/10% MeOH. This solution should be made weekly.

0.004 M Acetic Acid in 90/10 DCM/MeOH

Pipet 23 µL of Baker glacial acetic acid into a 100-mL volumetric flask and bring to volume with 90/10 DCM/MeOH. A new solution should be prepared weekly.

0.10 M Acetic Acid

Pipet 2.85 mL of Baker glacial acetic acid into 500-mL volumetric flask and bring to volume with Milli-Q® water. A new solution should be prepared weekly.

20% Acetonitrile/ 80% 0.10 M Acetic Acid

With 100-mL graduated cylinder, measure 100 mL of acetonitrile and add to a 500-mL volumetric flask. With a 500-mL graduated cylinder, measure 400 mL of 0.1 M acetic acid into the 500-mL volumetric flask. Do not adjust the volume to the 500-mL mark. Shake vigorously to mix. A new solution should be prepared weekly.

HPLC Eluents

Eluent A: 100% acetonitrile;
Eluent B: 100% pH 3, 30 mM phosphate buffer;
Eluent C: 100% Milli-Q® water.

Mobile phases should be thoroughly degassed daily. Solvents are degassed by filtering them through a Millipore® vacuum filtering apparatus while sonicating the apparatus. If a low-pressure mixing HPLC is used, mobile phases should be sparged at 30 mL/min.

Standards

Use Class A volumetric flasks when preparing standard solutions.

Stock Standard Solution

Prepare a standard stock solution by accurately weighing 10 mg of DPX-PE350 into a 100-mL volumetric flask on an analytical balance. *Record the weight of the standard used to make the stock solution.* Dissolve the standard in approximately 75 mL of HPLC-grade methanol. After dissolving, bring the solution to 100.00-mL volume using HPLC-grade methanol. This standard solution is stable for approximately 6 months when stored at approximately 4°C. The concentration of this solution is 100-µg/mL DPX-PE350 in methanol.

Intermediate Standard Solution

Prepare an intermediate standard solution by pipetting 1.00 mL of the 100-µg/mL DPX-PE350 stock standard into a 100-mL volumetric flask. Bring to volume using HPLC-grade methanol. The concentration of this solution is 1-µg/mL DPX-PE350 in methanol. This standard solution is stable for approximately 6 months stored at approximately 4°C.

Chromatographic Standard Solutions

The 1-µg/mL DPX-PE350 in methanol fortification solution is used to prepare the chromatographic standards. Prepare the standards by pipetting volumes of the 1-µg/mL intermediate standard solution of DPX-PE350 into a 25-mL volumetric flask, as shown in the following table:

Desired Standard Concentration (µg/mL)	Volume of 1 µg/mL Standard Required (mL)
0.500	12.5
0.250	6.25
0.200	5.00
0.100	2.50
0.0500	1.25
0.0250	0.625
0.0100	0.250
0.00500	0.125
0.00100	0.0250

Evaporate the methanol (to dryness) in each of the 25-mL volumetric flasks the using an N-Evap. Add 20% acetonitrile/80% 0.10 M acetic acid to the volumetric flasks and dilute to 25.00 mL. These standard solutions are stable for approximately 6 months at 40°C, but they should be prepared fresh weekly and stored at 4°C.

Fortification Standard Solutions

In most circumstances, the 1-µg/mL intermediate standard solution should be used for fortifications of samples analyzed by HPLC.

Analytical Procedure

Storage and Preparation of Water Samples

Water samples are stored at 4°C or frozen until analysis. Samples should be warmed to room temperature and thoroughly mixed before use one day before they are extracted.

Water Fortification Procedure

To fortify filtered water samples, add 200 mL of water to a 250-mL graduated cylinder. Add to the 200-mL water sample the required volume of fortification standard to achieve the appropriate fortification level (see table below). For HPLC method validation and quality control, samples can be fortified using the following table:

Volume of Standard (mL)	Standard Conc. (µg/mL)	Sample Volume (mL)	Fortification Level (ppb)
0.020	1.0	200	0.10
0.040	1.0	200	0.20
0.100	1.0	200	0.500
0.200	1.0	200	1.00
0.0100	100	200	5.00
0.0200	100	200	10.0
0.1000	100	200	50.0

Extraction Procedure

At a minimum, a sample set should consist of an unfortified and a fortified water sample. A sample set of six water samples, including unfortified and fortified samples, should be run. One, or more water samples may be extracted and cleaned up by doing the following:

1. Allow frozen or chilled water sample to come to room temperature. This step should be started the day before the extraction is to be done.
2. Filter each water sample through a 9-cm Whatman No. 3 filter paper using a clean Buchner funnel.
3. Using a clean, 250-mL graduated cylinder labeled with appropriate sample identification, measure 200 mL of sample water. *Record volume for sample.*
4. Fortify water sample (if applicable) by adding "X" µL of the 1-µg/mL DPX-PE350 intermediate standard solution to the water (see the table on the previous page for the amount added to the water). *Record the volume and concentration of the fortification standard used.*
5. Using a clean, 10-mL graduated cylinder, add 5-mL (± 1 mL) of 0.10 M formic acid in 90% DCM/10% MeOH to an Envi-Carb tube installed on the solid phase vacuum manifold. Pull the solution through the Envi-Carb tube at a 3-5 mL/min flow rate. Pull air through the tube for 5 min after the solution has passed through.
6. Rinse the 10-mL graduate used in Step 5 with HPLC grade acetone and discard the rinse to waste. *This graduated cylinder should be rinsed with acetone before it is used to measure a new rinse or elution solution for each of the following steps.*

7. Using the 10-mL graduate, add 7 mL (± 1 mL) of 0.1 M HCl to the Envi-Carb tube. Pull the solution through the Envi-Carb tube at a 3-5 mL/min flow rate. Pull air through tube for 2-3 seconds after 0.1 M HCl has passed through the packing.
8. Using the 10-mL graduate, add 5 mL (± 1 mL) of Milli-Q® water to the Envi-Carb tube and pull 2-3 mL through the packing at a flow rate of 3-5 mL/min.
9. Label the Envi-Carb tube with appropriate sample identification.
10. Attach a clean, Varian 1213-1030, 75-mL reservoir to the Envi-Carb tube using a Varian 1213-1003 adapter and add the 200-mL water sample to the reservoir. Pull the water sample through the Envi-Carb tube at a 3-5 mL/min flow rate. When the graduated cylinder is empty of sample water, rinse the graduate with approximately 10-mL Milli-Q® water and add to the reservoir. After all of the sample has passed through the reservoir, but not through the Envi-Carb tube, rinse the 75-mL reservoir with approximately 5 mL Milli-Q® water. Pull the final amount of water through the Envi-Carb packing until the first air bubble appears below the packing. Then, stop the flow and disconnect the reservoir and adapter from the Envi-Carb tube. Water pulled through the Envi-Carb tube goes to waste.
11. Using the 10-mL graduate, add 7 mL (± 0.1 mL) of 0.01 M ammonium carbonate to the Envi-Carb tube packing. Pull the solution through the packing at a 3-5 mL/min flow rate. Pull air through the packing for 1 min after the ammonium carbonate solution passes through the Envi-Carb tube. Solution pulled through the Envi-Carb tube goes to waste.
12. Using an autopipet, add 1.0 mL of methanol to the Envi-Carb tube. Pull the methanol through the packing at a flow rate similar to that in Step 11. Pull air through the packing for 5 min, after methanol passes through the Envi-Carb tube. Solution pulled through the Envi-Carb tube goes to waste.
13. Using the 10-mL graduate, add 6 mL (± 0.1 mL) of 4 mM acetic acid in 90% DCM/10% MeOH to the Envi-Carb tube. Pull the solution through the packing at a 3-5 mL/min flow rate. Pull air through the packing for 10-20 seconds after the solution passes through the Envi-Carb tube. Solution pulled through the Envi-Carb tube goes to waste.

18

14. Using the 10-mL graduate, add 5 mL (± 0.1 mL) of 0.10 M formic acid in 90% DCM/10% MeOH to the Envi-Carb tube. Pass the solution through the tube at a 3-5 mL/min flow rate, collecting the solution that passes through in a clean, appropriately labeled Pyrex, graduated, conical, centrifuge vial.
15. Evaporate the DCM/MeOH/formic acid solution to dryness in an N-Evap with the water bath at 40°C using nitrogen gas.
16. The sample may be stored in a capped, centrifuge vial, in a refrigerator at approximately 4°C for at least two weeks before analysis.
17. Bring sample up in 20% acetonitrile/80% 0.1 M acetic acid to a final volume of 1.00 mL. *Record the final volume used.* Vortex mix sample for 10 seconds, sonicate for 2 minutes, and vortex mix for 10 seconds. Filter sample using the Millipore SJHVL04NS HV, 0.45-µm filter connected to a plastic disposable syringe.
18. Analyze the sample using column-switching HPLC.

When possible, samples should be run from low to high concentration of DPX-PE350. For samples having unknown concentrations, sample vials should be loaded on an auto sampler in a nonsystematic fashion. Samples should be intermixed with standards (bracket every 2-3 samples with a standard). When possible, standard concentrations should be selected to bracket expected DPX-PE350 levels in the samples analyzed. A sample response should be within the responses of two standards analyzed in a sample set of water samples. A standard should be the first and last sample analyzed in each sample set.

At a minimum, a sample set should consist of an unfortified and a fortified water sample and six standards having concentrations that bracket the fortification level. A sample set of six water samples, including unfortified and fortified samples, should be run.

Before a set of chromatographic standards are used to determine the amount of DPX-PE350 in a water sample, they should be analyzed using HPLC to assure that they have been prepared properly. A 0.200-mL injection volume on the SB-CN column of each standard should generate a linear response from the UV detector set at 254 nm. Standards prepared from week to week should generate similar response factors. A six-point calibration curve should be used with standard concentrations in the range of the samples when possible.

Liquid Chromatography

The HPLC system components have already been described in the ***Equipment*** section of this report. Representative conditions for the HPLC method are shown in the following table:

Wavelength	254 nm
Column Temperature	40.0°C
Injection Volume	0.200 mL
HPLC Dwell Volume	4.5 mL
Mobile Phase A	100% ACN
Mobile Phase B	100% pH 3, 30 mM phosphate buffer
Mobile Phase C	100% Milli-Q® water

Multi-dimensional HPLC is used with the columns conditioned using the above procedure. (For a review of multi-dimensional, column-switching HPLC, see References 2 and 3.) A diagram of the column-switching valve arrangement is shown in Figure 1, where Column I and Column II are Zorbax® SB-CN and Zorbax® SB-C18 analytical columns, respectively. Tables I.A and I.B describe typical pump and column-switching timing sequences.

The following discussion describes the column-switching routine and how the technique is performed.

With the switching valve in Position 1, the effluent from Column I leaves the column through the switching valve, enters a bypass loop, flows back through the switching valve, and then flows to the detector. With the valve in Position 2, the effluent from Column I goes (via the valve) to Column II, back to the valve, and then to the detector. *All tubing connecting the switching valve to the analytical columns and detector should be 0.005-inch internal diameter tubing to minimize dead volume.* Either stainless steel or PEEK tubing can be used.

Before injection, the valve is put in Position 1, so that the HPLC flow bypasses Column II. Pump 20% ACN/80% pH 3, 30 mM potassium phosphate buffer at 1.0 mL/min through Column I only. A linear gradient is run from 20 to 43% acetonitrile in five minutes. When DPX-PE350 starts to elute from Column I, the valve is switched to Position 2 in order to trap the peak on Column II. After the peak is collected at the head of Column II, the valve is switched back to Position 1.

The switching valve time (the "window of transfer", or "time window", or "cut window") is determined immediately before the analysis of a sample set is started. Determine the time window by injecting a DPX-PE350 standard three times (a 250-ng/mL DPX-PE350 standard can be used) **on Column I only**. Run a

mobile phase linear gradient from 20 to 43% acetonitrile in five minutes at 1 mL/min for each injection. **The column temperature should be checked to confirm that the column oven is working.** Calculate the mean retention time of the three DPX-PE350 standard injections. Set the switching valve time window at ± 0.25 minutes of the mean retention time for DPX-PE350.

Typical DPX-PE350 baseline peak widths for 0.250-µg/mL standards injected should range from 0.4 to 0.6 min, depending on the SB-CN guard and analytical columns used. The retention time (through Column I) relative standard deviation (RSD = 100 * Std. Dev./Mean) for the standards injected should be no greater than 1%. In some circumstances, depending on the HPLC instrumentation, premixed solvents may be required to generate reproducible retention times.

When the mean retention time is being determined for standards eluting from Column I, the peak shape of DPX-PE350 should be observed. If the peak tails badly or recoveries are poor, the pre-column and/or analytical column may need to be replaced before further analysis.

If new analytical columns are to be used or if columns have not been used for a day or more, they should be conditioned using the following procedure:

A 100% ACN mobile phase is passed through both columns at 1 mL/min. The baseline is monitored during this process until a stable baseline is observed.

Next, the columns are conditioned in the mobile phases that are used for the sample analysis. Column II (the SB-C18 column) is conditioned with 43% acetonitrile/57% pH 3, 30 mM potassium phosphate buffer for 30 min at 1 mL/min by passing the mobile phase through both columns. Then, the valve is switched to Position 1 and Column I is conditioned with 20% acetonitrile/80% pH 3, 30 mM potassium phosphate buffer for 5 min at 2 mL/min.

After conditioning the columns, the auto sampler is purged with 20% acetonitrile/80% pH 3, 30 mM potassium phosphate buffer for 3 min at 1 mL/min.

Injection of several water samples into the guard (pre-column) and SB-CN analytical column may affect the peak shape and retention time of DPX-PE350. If peak tailing is observed when standards elute from the SB-CN column, the SB-CN guard column (pre-column) is replaced. If peak

tailing continues after changing the guard column, then the analytical column should be replaced.

When a pre-column is replaced, it is purged with 100% ACN for 5 min at 2 mL/min before connecting the pre-column to the analytical column. After conditioning the pre-column, the pre-column and SB-CN analytical column are purged with 100% ACN for 5 min at 2 mL/min and the column oven chamber is reequilibrated to 40.0°C. Then, the pre-column and analytical column are conditioned with 20% ACN/80% pH 3, 30 mM potassium phosphate buffer for 5 min at 2 mL/min.

The following column-switching routine is used to separate DPX-PE350 from coextracted compounds (**see Figure 1 and Tables I.A and I.B**). A 0.20-mL sample is injected into Column I. The initial mobile-phase concentration is 20% ACN/80% pH 3, 30 mM potassium phosphate buffer at a flow rate of 1 mL/min. The solvent delay time is about 1.8 min on Column I.

At the beginning of the time window (the time window is approximately 10 minutes from the point of injection), the valve is switched from Position 1 to Position 2 and DPX-PE350 is transferred to Column II. At the end of the time window, the valve is switched from Position 2 to Position 1. The mobile phase gradient is allowed to finish on Column I and after completion of the gradient, Column I is maintained at 43% acetonitrile/57% pH 3, 30 mM potassium phosphate buffer to clean out the column.

After cleaning Column I, the valve is switched to Position 2, to elute DPX-PE350 from Column II using the 43% ACN/57% pH 3, 30 mM potassium phosphate buffer mobile phase. DPX-PE350 elutes from Column II at a retention time of about 24 min from the start of the run. After DPX-PE350 elutes from Column II, 75% ACN/25% Milli-Q® water is passed through both columns at 1 mL/min for 15 min to more rigorously clean off Column I and to clean off Column II.

After cleaning off the columns, they are conditioned at their initial conditions. To accomplish this, a 43% ACN/57% pH 3, 30 mM potassium phosphate buffer is passed through both columns at 1.3 mL/min to set Column II at the conditions required for the next separation. Then, the valve is switched to Position 1 and 20% ACN/80% pH 3, 30 mM potassium phosphate buffer is passed through Column I only at 2 mL/min for 5 min. The flow rate is reduced to 1 mL/min and the system is allowed to run for another one minute. At this time, Column I and Column II are both ready for the next injection.

22

Calculations

Trace levels of DPX-PE350 found in water samples are determined using the procedure discussed below.

Generate a peak height (μV) *vs.* known concentration (ng/mL) plot of external DPX-PE350 standards injected. Generate a linear least squares fit calibration curve for the data. The equation for the line is $y = mx + b$, where y is the peak height, x is the concentration of DPX-PE350 (ng/mL), m is the slope of the line (μV/ng/mL), and b is the y, (ordinate) intercept (μV). The solution to the equation for this line gives the concentration of DPX-PE350 found (ng/mL) corresponding to the experimentally observed peak height (μV).

Using the experimentally observed peak height to determine the concentration found from the calibration curve, the ppb DPX-PE350 found in a water sample is calculated.

ppb found

The parts per billion (ppb) DPX-PE350 found in water samples is given by:

$$ppb \text{ Found} = \frac{(1000 \text{ ng}/\mu\text{g})(\text{Conc.Found, } \mu\text{g}/\text{mL})(\text{Final Vol., mL})}{\text{Sample Volume, mL}}$$

where Conc.Found is the concentration found and Final Vol. is the final volume of the sample.

Fortification Level (ppb)

The fortification level (ppb) is the amount of DPX-PE350 added to 200 mL of water sample which is given by:

$$\text{Fort. Level, ppb} = \frac{(1000 \text{ ng}/\mu\text{g})(\text{VFS, mL})(\text{CFS, mL})}{\text{Sample Volume, mL}}$$

where Fort. Level is the fortification level, VFS is the volume of the fortification standard that was added to the water, and CFS is the concentration of the fortification standard.

Recovery

The recovery is given by

$$\% \text{ Recovery} = 100\left(\frac{\text{ppb found}}{\text{Fortification level, ppb}}\right)$$

Sample Calculations

Using the data for NC 100ppt2/6-23, North Carolina, water sample that was extracted June 23, 1994 (see Data Sheet 6 in Appendix I), the following sample calculations were prepared:

$$ppb \text{ Found} = \frac{(1000 \text{ ng}/\mu\text{g})(0.0200 \ \mu\text{g}/\text{mL})(1.00 \text{ mL})}{200 \text{ mL}} = 0.100 \text{ ppb}$$

$$\text{Fort. Level} = \frac{(1000 \text{ ng}/\mu\text{g})(0.0200 \text{ mL})(1.00 \ \mu\text{g}/\text{mL})}{200 \text{ mL}} = 0.100 \text{ ppb}$$

$$\% \text{ Recovery} = 100\left(\frac{0.100 \ \mu\text{g}/\text{mL}}{0.100 \ \mu\text{g}/\text{mL}}\right) = 100$$

Cleaning Procedures

All glassware can be cleaned by means of any approach that is consistent with trace organic analysis.

Generally, the following is done. Glass items are initially rinsed with acetone (technical grade), followed by a thorough scrubbing with an aqueous soap solution (prepared in tap water). Then, glassware is rinsed with tap water, followed by another acetone rinse.

Detector Response

DPX-PE350 absorbs ultraviolet light at 254 nm. This wavelength is used in this method. The UV detector response at 254 nm was linear over the range of standards analyzed, 10-250 ng/mL (see Figure 2).

TABLES I.A AND I.B
TYPICAL HPLC PUMP AND COLUMN-SWITCHING TIMING SEQUENCE

Table I.A. Times and values for column switching

#	Time (min)	Event	Function	Explanation
1	0.00	Event 3	On	Start run through Column I only
2	0.00	Event 4	Off	
3	10.26	Event 4	On	Start column switch; DPX-PE350 is transferred
4	10.26	Event 3	Off	
5	10.76	Event 3	On	End-column switch; Clean Column I
6	10.76	Event 4	Off	
7	15.00	Event 4	On	Start analytical separation on Column II
8	15.00	Event 3	Off	
9	44.90	Event 3	On	Set Column I to initial conditions
10	44.90	Event 4	Off	

The Waters pump control module has four external contact closure (TTL to GND) events that are activated using the Millennium 2010 software. The values of Event 3 and Event 4 (on and off times) control the Valco column switching valve: Event 3 off, Event 4 on = valve in Position 1; Event 3 on, Event 4 off = valve in Position 2. The Valco valve wiring is hooked up in the following way to the pump control module: red-coated wire to Event 3-slot 5, black-coated wire to Event 4-slot 7, and green-coated wire to Event 3-slot 6. A jumper wire connects Event 3-slot 6 to Event 4-slot 8. If both events are turned on at the same time, the valve continues to rotate; therefore, flow through the system stops.

Table I.B. Times and values of mobile-phase gradients and flow rate using the Waters Pump Control Module (PCM)

#	Time (min)	Flow (mL/min)	%A	%B	%C	Curve Type	Explanation
1	0.00	1.00	20.0	80.0	0.0	0	Start linear gradient on Column I only
2	5.00	1.00	43.0	57.0	0.0	6	Isocratic conditions to clean off Column I
3	25.00	1.00	75.0	0.0	25.0	11	Clean off Columns I and II
4	30.00	1.30	43.0	57.0	0.0	11	Set Column II at initial cond.
5	45.00	2.00	20.0	80.0	0.0	11	Set Column I at initial cond.
6	50.00	1.00	20.0	80.0	0.0	11	Set at initial flow rate

Curve Type 0 on the Waters HPLC system is the starting condition for the analysis. Curve Type 6 on the Waters HPLC system is a linear gradient that starts at the time listed on line 1 and ends at the time listed on line 2. Curve Type 11 on the Waters HPLC system is a step gradient that begins at the time listed on the line that the curve is specified. Mobile phases A, B, and C are 100% ACN and 100% pH 3, 30 mM potassium phosphate buffer, and Milli-Q® water, respectively.