

ANALYTICAL METHOD (COLUMN SWITCHING/HEART CUT) FOR THE DETERMINATION OF RESIDUES OF CHLORIMURON ETHYL (DPX-F6025) IN SOIL

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ABSTRACT

An analytical method based on the use of multi-dimensional HPLC with UV absorbance detection at 240 nm was developed for the determination of chlorimuron ethyl (DPX-F6025) in soil. The limit of detection for chlorimuron ethyl in soil is 0.200 ppb, the limit of quantitation is 0.400 ppb.

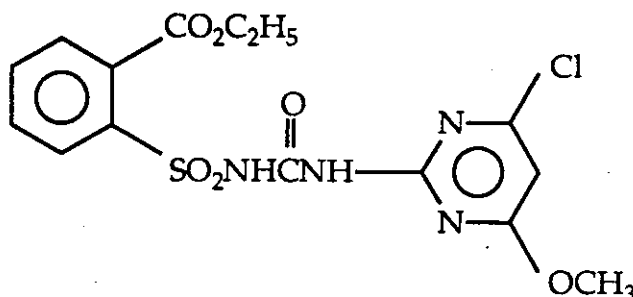
The isocratic HPLC method (one HPLC, one pump, and one switching valve) employs two columns in series. The first column is used as a "clean-up" column from which chlorimuron ethyl is transferred (heart-cut) to the second column. The transfer occurs during a ± 0.2 -minute window in relation to the peak retention time (apex) of chlorimuron ethyl. Once the analyte is on the second column, the analytical separation of chlorimuron ethyl occurs.

INTRODUCTION

The analytical method presented within was developed to detect chlorimuron ethyl residues in soil. An isocratic multi-dimensional HPLC method with UV absorbance at 240 nm is employed.

The soils analyzed are typical of chlorimuron ethyl use areas. Characterization data are found in Tables VII-X.

Chlorimuron ethyl (DPX-F6025) is a sulfonylurea herbicide with the structure as seen below and a pK_a value of 4.2.



DPX-F6025

Ethyl 2-[[[(4-chloro-6-methoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate

CAS Registry Number: 90982-32-4

The fortified and unfortified soil matrix extracts were cleaned up prior to injection onto the HPLC system by passing the sample through a pre-treated extraction disk - C8 Empore™. The Empore™ extraction disk is employed since separation can occur more quickly than with conventional solid-phase extraction cartridges/columns (e.g., Bond Elut®).

Chlorimuron ethyl is eluted from the disk with 100% ethyl acetate. The solution is then evaporated to dryness and resuspended in the initial mobile phase (35% ACN/65% 0.03 M phosphate buffer pH 3.0). A 200- μ L sample is injected on a Zorbax® SB-Cyano column. At ± 0.2 minutes of the peak retention time (apex) for chlorimuron ethyl on the SB-Cyano column, the valve is switched, and chlorimuron ethyl is loaded and concentrated onto the second column - a Zorbax®-ODS column. This type of column switching is termed "heart cutting". Once the first column is cleaned and equilibrated to the mobile phase of the second column, the analytical separation occurs with both columns in series.

Method validation consists of analysis of three (3) control soil samples and three (3) fortified soil samples at each of the following levels: LOD (0.200 ppb), 2.5x LOD, LOQ (0.400 ppb), and 4-6x LOD (1.00 ppb). The analysis of replicates was performed on separate days.

Method validation on Bayboro consists of two (2) fortified samples at 0.200 ppb, two (2) at 0.400 ppb, and one (1) at 1.00 ppb, due to a lack of control soil.

EXPERIMENTAL

Equipment

Equivalent equipment may be substituted.

Liquid Chromatograph

Waters HPLC System #42350 (Millipore, Inc., Bedford, Mass.)

System Controller, Waters Model 600E

Fluid Unit, Waters Model 600

Photodiode Array Detector, Waters Model 991

Powerline V6.22A run on a NEC Powermate 386/25 Computer

Autoinjector with Heating and Cooling System, Waters Model 717 WISP #78505

Temperature Control Module #38039, Waters

Column Heater Module #38040, Waters

6 port VICI external switching valve, Model E60, #EC6W (Valco Instruments Inc., Houston, Tex.)

HPLC Columns

Pre-Column 1 Zorbax® SB-CN 4.0 x 12.5 mm, 5-micron Reliance Cartridge Guard Column, #820674-916 and column end-fittings, #820529-901 (MAC-MOD Analytical Inc., Chadds Ford, Pa.)

Column 1 Zorbax® SB-CN 4.6 x 150 mm, 5-micron analytical column, #883975-905 (MAC-MOD Analytical Inc., Chadds Ford, Pa.)

Column 2 Zorbax® ODS 4.6 x 150 mm, 5-micron
analytical column, #883952-702 (MAC-MOD
Analytical Inc., Chadds Ford, Pa.)

OR

Zorbax® ODS 4.6 x 250 mm, 5-micron
analytical column, #880952-702 (MAC-MOD
Analytical Inc., Chadds Ford, Pa.)

Empore™ Station

Six place Solid-Phase Extraction Manifold Kit, 47 mm,
#XX0047SPE (Millipore, Bedford, Mass.)

Vacuum Controller

Vacuum Controller/Release #1223-4002 (Varian Sample
Preparation Products, San Fernando, Calif.)

Solid-Phase Extraction Disk

C8 Empore™, 47-mm disks, #1214-5002 (Varian Sample
Preparation Products, San Fernando, Calif.)

Empore™ Flask Setup (Millipore, Bedford, Mass.)

Filter Reservoir 300 mL #XX1004704

Glass Filter Base #XX1504702

Glass Flask, 1 L, #XX1504705

Anodized Aluminum Spring Clamp #1004703 or

All-Glass Filter Holder, 47 mm #XX1504700

Evaporator

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

Zymark® TurboVap® 500 Closed Cell Concentrator, #ZW500
(Zymark® Corporation, Hopkinton, Mass.)

Zymark® Concentration sample tube-1.0 mL stem, #ZA7527
(Zymark® Corporation, Hopkinton, Mass.)

Separatory Funnel

KIMAX® 500-mL Pear-Shaped Separatory Funnel (Kimble #29048F-500) (VWR Cat. No. 30352-222, VWR Scientific, Bridgeport, N.J.)

Filters

Solvents/Buffers	Nylaflo™ 0.45-micron, 47-mm nylon discs, Part No. 66608 (Gelman Sciences, Ann Arbor, Mich.)
Samples prior to Empore™	MF-Millipore Membrane Filters, 1.2-micron, 47-mm disks, Part No. RAWP04700 (Millipore, Inc., Bedford, Mass.)
	Durapore-Millipore Membrane Filters, 0.65-micron, 47-mm disks, Part No. DVPP04700 (Millipore, Inc., Bedford, Mass.)
Samples prior to HPLC	Non-Sterile Millex-HV13, 0.45-micron, 13-mm disposable filter unit, Part No. SJHV013NS (Millipore, Inc., Bedford, Mass.)

Centrifuge

DuPont Sorvall® Model RC5C (DuPont Instruments, Wilmington, Del.)

Centrifuge Rotor

DuPont Model H2000B, HS-4 (38 cm, diameter) (DuPont Instruments, Wilmington, Del.)

Centrifuge Bottles

250 mL, polypropylene copolymer, "Wide Mouth" Nalgene®, VWR #21010-590 (VWR Scientific, Bridgeport, N.J.)

Homogenizer

Tekmar® SKT Tissumizer® Model SDT-1810 with a Model SDT-182EN shaft and generator (Tekmar Co., Cincinnati, Ohio)

Test Tubes

For Empore™ Station:

20 x 150 (OD, mm x Length, mm) Kimax® Chemical Test Tube, Part No. 60809-167 (VWR Scientific Co., Bridgeport, N.J.). The test tubes were cut down to 130 mm in length by the DuPont glass shop.

Syringe

For HPLC Sample:

2.5-mL disposable plastic syringe "Fortuna", Part No. Z11685-8 (Aldrich Chemical Co., Milwaukee, Wis.)

pH Meter

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

pH Buffer Standards

Buffer Solution pH 4.0, Part No. 34170-106 (VWR Scientific Co., Bridgeport, N.J.)

Buffer Solution pH 7.0, Part No. 34170-115 (VWR Scientific Co., Bridgeport, N.J.)

Buffer Solution pH 10.0, Part No. 34170-124 (VWR Scientific Co., Bridgeport, N.J.)

Balances

Mettler AC100 and Mettler PM600 (Mettler, Hightstown, N.J.)

Ultrasonic Bath

Branson Model 2210 ultrasonic bath (VWR Cat, No. B2210DTH, VWR Scientific Co., Bridgeport, N.J.)

Mixer

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

Reagents and Standards

Equivalent reagents may be substituted for any of those listed below.

Water

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

50% (w/w) Sodium Hydroxide (NaOH)

#3727-01 (J. T. Baker, Phillipsburg, N.J.)

Ethyl Acetate

EM Omni Solv #EX0241-1 (EM Science, Gibbstown, N.J.)

Hexane

EM Omni Solv #HX0296-1 (EM Science, Gibbstown, N.J.)

Methanol

EM Omni Solv #MX0488-1 (EM Science, Gibbstown, N.J.)

Acetonitrile (ACN)

Fisher OPTIMA #A996-4 (Fisher Scientific, Fair Lawn, N.J.)

Potassium Phosphate, Dibasic (K₂HPO₄)

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

Potassium Phosphate, Monobasic (KH₂PO₄)

"Baker Analyzed Reagent" #3246-05 (J. T. Baker, Inc., Phillipsburg, N.J.)

Phosphoric Acid (H₃PO₄), Concentrate

"Baker Analyzed Reagent" #0260-01 (J. T. Baker, Inc., Phillipsburg, N.J.)

Chlorimuron ethyl

Reference substance used to prepare standard solutions.
Analytical Standard grade (DPX-F6025, Lot #113, 99.81% pure)
(DuPont Agricultural Products, Global Technology Division,
E. I. du Pont de Nemours and Company).

Preparation of Solutions

0.03 M Phosphate Buffer pH 3.0

Add 3.61 g of KH_2PO_4 to 1 L of Milli-Q® water. Adjust pH to 3.0 (± 0.1) using concentrated H_3PO_4 .

0.10 M K_2HPO_4 (not pH adjusted)

Dissolve 17.42 g of K_2HPO_4 in 800 mL of Milli-Q® water and dilute to 1 liter (do not pH adjust, but record pH).

50% Phosphoric Acid (v/v)

Add 50 mL of concentrated phosphoric acid to 50 mL of Milli-Q® water and mix.

35% ACN:65% 0.03 M Phosphate Buffer, pH 3.0 Solution

Add 35 mL of ACN to 65 mL of 0.03 M phosphate buffer. Make solution fresh every other week.

Eluents for the HPLC System:

Eluent A

100% 0.03 M Phosphate Buffer, pH 3.0 (± 0.1)

Eluent B

100% acetonitrile (ACN)

Eluent C

100% Milli-Q® water

Eluent D

100% Methanol

Standards:

Stock Standard Solution

Accurately weigh 0.010 g of chlorimuron ethyl and dissolve in 100.0 mL of acetonitrile to make a 100- $\mu\text{g}/\text{mL}$ stock standard. Make this standard stock solution fresh weekly and store at 4°C.

20 $\mu\text{g}/\text{mL}$ Intermediate Standard

Dilute 2.0 mL of stock standard solution to 10 mL with acetonitrile.

200-ng/mL Intermediate Standard

Dilute 100 μL of the 20- $\mu\text{g}/\text{mL}$ solution to 10 mL with acetonitrile.

Chromatographic Standard Solutions

Make chromatographic standard solutions daily and directly prior to the start of the HPLC run. Measure the stock standard solution, ACN, and buffer with a Pipetman (Gilson Scientific) and prepare as follows:

<u>Chromatographic Standard Concentration</u> $\mu\text{g}/\text{mL}$	<u>Stock Standard Solution Volume</u> mL (μL)	<u>Stock Standard Solution Concentration</u> $\mu\text{g}/\text{mL}$	<u>Volume of ACN</u> mL	<u>Volume of Phosphate Buffer</u> mL	<u>Final Volume</u> mL
0.10	0.01 (10)	100	3.49	6.5	10
0.20	0.02 (20)	100	3.48	6.5	10
0.50	0.05 (50)	100	3.45	6.5	10
1.0	0.10 (100)	100	3.40	6.5	10
1.5	0.15 (150)	100	3.35	6.5	10

All chromatographic standards (and extracted samples) are in the initial mobile phase (65:35 0.03 M phosphate pH 3.0:ACN), and are ready for injection into the HPLC.

Use chromatographic standards the day they are prepared and discard after analysis.

Fortification Standard Solution

Use the 200 ng/mL intermediate standard solution for all fortifications.

Analytical ProcedureStorage and Preparation of Samples

Control samples are obtained from DuPont Ag Products and stored at 4°C. Thoroughly mix samples before analysis. Characterization data are found in Tables VII-X.

Fortification Procedure (Soil)

Fortify 100-g samples of soil with an appropriate amount of the fortification standard solution (chlorimuron ethyl in ACN). For method validation, samples are fortified at a level shown in the following table:

<u>Volume of standard (mL) [μL]</u>	<u>Standard Concentration (ng/mL)</u>	<u>Sample Weight (g)</u>	<u>ppm Fortified (ng/g)</u>
0.50 [500]	200	100	1.0
0.20 [200]	200	100	0.40
0.10 [100]	200	100	0.20

After fortification, let the samples air dry for at least 15 minutes.

Empore™ Setup

The Empore™ elution apparatus and the vacuum arrangement are shown in Figure 1. The pressure settings used during conditioning, pre-wetting, sample addition, and sample elution are listed in Table I.

Several precautions must be observed when using the Empore™ disks:

- When fortifying matrix with the intermediate standard solutions, a minimum amount of ACN must be used since breakthrough of chlorimuron ethyl from the EMPORE™ disk will occur. Maximum concentration of ACN that can be present is 30% with a final extract volume of at least 200 mL.

- The final Empore™ elution volumes (3 x 5 mL) that are stated in the extraction procedure are the minimum volumes that can be used. If the final Empore™ volumes are reduced, the percent recoveries will decrease.
- It should take 8-10 minutes to filter the sample through the Empore™ disk. Longer times are acceptable but times less than 5 minutes will result in break-through of the analyte (some of the chlorimuron ethyl will not be retained on the Empore™).
- During pre-wetting and sample addition stage, the Empore™ disk cannot go dry. If this should occur, recondition the disk and continue to the pre-wetting stage.

Extraction Procedure (Soil)

1. Weigh 100.0 g (\pm 0.1 g) of soil into a 250-mL centrifuge bottle.
2. Fortify, if needed, by adding the fortification volume of a 200-ng/mL stock solution to each bottle (see table on previous page).
3. Let the fortified matrix sit for 15 minutes.
4. Add 25 mL of 0.03 M Phosphate buffer pH 3.0.
Add 100.0 mL (\pm 1 mL) of 100% ethyl acetate to each centrifuge bottle and mix.
5. Let the sample soak for ten minutes.
6. Shake the sample continuously for 15 minutes.
7. Centrifuge the sample for 10 minutes at 3000 RPM (2,800-4,200 g) at 4°C.
8. Decant off and combine each samples supernatant in a labeled TurboVap Flask.
9. To the pellet, add 25 mL of 0.03 M Phosphate buffer pH 3.0 and 100 mL of 100% ethyl acetate. Shake by hand to break up the pellet. Repeat Steps 6 and 7 and continue with Step 10.
10. Combine supernatants into the labeled TurboVap Flask.
11. To each flask add 100 mL of 0.1 M K_2HPO_4 (not pH adjusted).

12. Evaporate using a TurboVap at 40°C until 80-90 mL remains; 20 min at 6000 rpm, 20 min at 7000 rpm, and 20 min at 8000 rpm fan speed.
13. Transfer quantitatively to a labeled 500-mL separatory funnel.
14. Rinse flask with two (2) 50-mL portions of 0.1 M K_2HPO_4 (not pH adjusted) and add this to the flask.
15. Add 50 mL of hexane, shake vigorously with venting; allow to separate and drain bottom layer into a labeled centrifuge bottles, discard top hexane layer.
16. Transfer sample back into the separatory funnel and repeat Step 15.
17. Using a pH meter, adjust the pH (the sample must be at room temperature) of each aqueous extract to 5.0 ± 0.1 with 50% (v/v) H_3PO_4 .
18. Centrifuge the sample for at least 20 minutes at 4°C at a minimum of 7,000 rpm (6,000-10,000 g).
19. Filter each sample with a 1.2- μ m MF-Millipore filter on top of a 0.65- μ m Durapore-Millipore membrane filter.
20. Condition a C8 Empore™ extraction disk by passing 20.0 mL of ethyl acetate through it and air dry the disk for 5 minutes.
 - Rinse with 20-mL MeOH (do not let the disk go dry).
 - Rinse with 20 mL of Milli-Q® water, followed by 20.0 mL of 0.03 M phosphate pH 5.0 buffer.
21. Pass all of the filtered sample (from step #19) through the C8 Empore™ extraction disk and let the disk air dry for 15 minutes.
22. Collecting in a test tube, elute DPX-F6025 off the C8 Empore™ extraction disk with three 5-mL portions of 100% ethyl acetate. Let each of the 5-mL fractions pass completely through the Empore™ before the addition of the next 5-mL fraction.
23. Evaporate the sample to dryness under a stream of nitrogen at room temperature.

24. Dilute all samples to 1.0 mL with a 35% ACN/65% 0.03 M Phosphate, pH 3.0 buffer. Sonicate/Vortex sample prior to use.

HPLC Sample Preparation

Immediately prior to HPLC analysis, transfer the sample to a Fortuna® syringe barrel fitted with a Millex-HV® filter unit. Insert the syringe plunger and filter the samples into HPLC autosampler vials. Dispose of the remaining (unfiltered) portion of each sample. Inject samples on the HPLC, intermixed with standards (bracket every 2-3 samples with a standard); load vials on the autosampler in a nonsystematic fashion. Select the levels of standards to surround the expected chlorimuron ethyl levels (if known) in the samples analyzed.

Liquid Chromatography

The HPLC system is described in the **Equipment** section. Under conditions described below, chlorimuron ethyl exhibits a retention time of about 54 min for soil. A typical chromatogram of a fortified chlorimuron ethyl sample can be seen in Figure 2.

Typical conditions for the HPLC method are presented in the table below:

Column I	
Drummer	15-cm Zorbax® SB-Cyano column
Tama	15-cm Zorbax® SB-Cyano column
Commerce	15-cm Zorbax® SB-Cyano column
Bayboro	15-cm Zorbax® SB-Cyano column
Column II	
Drummer	15-cm Zorbax® ODS column
Tama	15-cm Zorbax® ODS column
Commerce	15-cm Zorbax® ODS column
Bayboro	25-cm Zorbax® ODS column
Wavelength	240 nm
Column Temperature	35°C
Injection Volume	150 µL
Auto Sampler Temperature	4°C
Mobile Phase A	65%:35% 0.03 M Phosphate pH 3.0:ACN
Mobile Phase B	20%:80% 0.03 M Phosphate pH 3.0:ACN
Mobile Phase C	55%:45% 0.03 M Phosphate pH 3.0:ACN
Chart Speed	5 mm/min
Spurge*	Helium

* For the first 30 min of a run, helium spurge was set at 100 mL/min; after 30 min, the helium spurge was set to 50 mL/min.

Isocratic multi-dimensional HPLC is used with the combination of columns seen in Table II. (For a review of multi-dimensional, column-switching HPLC, see References 1 and 2.) The Zorbax® SB-Cyano column is used in the Column I position since chlorimuron ethyl was retained longer on this column than the majority of coextractives under these conditions.

If needed, a longer SB-ODS column (25 cm) can be employed so that greater separation of chlorimuron ethyl from coextractives can be achieved. With a longer column (25 cm), many of the matrix interference peaks observed with the 15-cm column are resolved.

A Zorbax® ODS column is used in the Column II position since chlorimuron ethyl is strongly retained, resulting in a relatively narrow peak width upon elution. See **Special Precautions** for a discussion of steps required prior to initial use of the listed HPLC columns.

A diagram of the columns and switching-valve arrangement is shown in Figure 3, where the first column (Column I) is the Zorbax® SB-Cyano column, and the second column (Column II) is a Zorbax®-ODS Column. In valve position 1, the eluate from Column I goes through a 1- μ L bypass loop, back to the valve, and then to the detector. In position 2, the eluate from Column I goes (via the valve) to Column II, back to the valve, and then to the detector.

Presented in Table III are typical timing sequences for analysis of soil. The Waters Powerline program controls the sequence of operations. The column-switching program (COLSW2) first equilibrates the columns (CSSTART) to the correct mobile phase. The second part of the program (CS1) analyzes the sample while the third part of the program (FINAL) cleans the columns, stores the columns in methanol, and turns off the pump and the helium sparge.

The following is an outline of what occurs at each time point during the column-switching program (CS1).

1. At the time of injection, put the valve in position 1, so that the HPLC flow bypasses Column II.
2. Pump mobile phase A at 1.0 mL/min through Column I only.

3. When chlorimuron ethyl starts to elute from Column I, switch the valve to position 2, to trap the peak on Column II. The valve-switching times (the "time window") are set at ± 0.20 minutes around the peak retention time (apex) for chlorimuron ethyl in soil on Column I as determined each day. *If greater selectivity is desired (due to an interfering peak, for example), the time window can be set to -0.15 to +0.15 min with respect to the retention time for chlorimuron ethyl.*
- The time window is determined each day prior to the sample analysis run (within two hours of the analysis run). The peak retention times (apex) for chlorimuron ethyl on Column I are determined by running a 0.5-ppm standard three times and determining the average retention time. This average peak retention time is then used to determine the time window. The peak retention time find program is run by the Waters Powerline using the COLSW1 program which is presented in Table IV.
 - If the relative standard deviation of the average peak retention time is greater than 1.0%, a new 0.5-ppm standard is prepared and the peak retention time program is run again. If this does not help to reduce the standard deviation, the Zorbax® SB-CN is replaced.
 - If peak tailing is observed during the peak retention time find program, the SB-CN pre-column is replaced and the column oven chamber is reequilibrated to 35°C for 30 min with 100% Milli-Q® water running through the system. After the system is equilibrated, the peak retention time program is run again and the average peak retention time is determined.
 - The selection of time window is also dependent on the Column I selection. The CN column retains less matrix than other reverse-phase columns, but the peak retention time for chlorimuron ethyl is maintained throughout an entire set of sample analyses with greater precision; this permits use of narrower time windows. Also, the SB-CN column requires less organic solvent in the mobile phase to elute

chlorimuron ethyl than with other reverse-phase columns; therefore, when chlorimuron ethyl is transferred to the ODS column, chlorimuron ethyl is retained strongly and the peak is "focused" at the head of the column.

4. After the peak has been collected at the head of Column II, switch the valve back to position 1.
5. After chlorimuron ethyl has been trapped on Column II, the mobile phase is changed from A to B, and the flow rate is increased to 2.0 mL/min, to quickly clean off Column I (a 5-6 min wash is needed).
6. The mobile phase is then changed to mobile phase C at a high flow rate (2.0 or 3.0 mL/min) through Column I only to equilibrate Column I to the new mobile phase.
7. After Column I has been reequilibrated (usually 10-15 min is required; this step should be carried out until no additional eluting peaks are observed), decrease the flow rate to 1.0 mL/min for 5 minutes. Now Column I is in the correct mobile phase to complete the analytical separation on Column II.
8. At this time, the valve is switched to position 2, to elute chlorimuron ethyl from Column II.
9. After baseline has been achieved, the valve is switched to position 1 and Column I is equilibrated to mobile phase A at high flow rate (2.0 or 3.0 mL/min) for 10 min, to hasten the equilibration process.
10. The flow rate is reduced to 1 mL/min and the system is allowed to run for another 5 min. At this time, Column I and Column II are ready for the next injection.

Calculations

Chromatographic peak heights were measured by the Powerline integration method. The integration settings are as follows:

Chromatogram Chart	
Partitions	1
Paper Speed	5 mm/min
Y-Scale	-0.05 - 0.05 AU/FS
Correction Constant	1
Smoothing	7 points
Slope	0.005 AU/min
Drift	0.002 AU/min
Height	0.001 AU
Width	0.02 min
Minimum Area	0.0001 AU/min
Time Double	30 min
Minus Peak	OFF

Response Factor (RF)

The response factor (RF) is the ratio of the detector response [peak height in AU (absorbance units)] to the concentration of chlorimuron ethyl injected (ppm, $\mu\text{g}/\text{mL}$). A standard should be run before and after every group of 2-3 samples. The average RF from all the standard chromatograms is calculated and used to determine the chlorimuron ethyl concentration in each sample.

$$\text{RF} = \frac{(\text{peak height, AU})}{(\text{DPX-F6025 standard ppm injected, } \mu\text{g/mL})}$$

Standard deviation (SD) and relative standard deviation (RSD) are calculated for the average response factor. If the RSD (for the 4 standards) is greater than 20%, the analysis run is not used.

Fortification Level (FL)

$$\text{FL} = \frac{(\text{SV})(\text{SC})}{\text{SW}}$$

where

SV = Volume of the intermediate standard that was used
 SC = Concentration of intermediate standard
 SW = Sample weight

Example (From data sheet Soil2697-1 seen in Appendix I)

$$\begin{aligned}
 SV &= 0.100 \text{ mL} \\
 SC &= 200. \text{ ng/mL} \\
 SW &= 100. \text{ g} \\
 FL &= \frac{(0.100 \text{ mL})(200. \text{ ng/mL})}{100. \text{ g}} \\
 &= 0.200 \text{ ng/g} = 0.200 \text{ ppb}
 \end{aligned}$$

Concentration of the Analyte in the Sample (ppb found)

ng/g or ppb chlorimuron ethyl in the sample is equal to the following:

$$\text{ppb} = \frac{(\text{PH})(\text{FV})}{(\text{ARF})(\text{SW})} \left[\frac{\text{IV}}{\text{EV}} \right]$$

where

- PH = Peak height
- FV = Final volume of sample that has been extracted prior to injection in the HPLC
- ARF = Average response factor for a given set of data (described above)
- SW = Sample weight
- IV = Initial volume
- EV = Empore™ volume (volume of extract that was added during the Empore™ step)

(the ratio of IV to EV for this study was always 1)

Example (from data sheet Soil2697-1 seen in Appendix I)

$$\begin{aligned}
 \text{ppb found} &= \\
 &= \frac{[(0.0004 \text{ AU})(1.00 \text{ mL})]}{[(1.83 \text{ E-}05 \text{ AU}/\mu\text{g/mL})(100. \text{ g})]} ([200 \text{ mL}/200 \text{ mL}]) \\
 &= 0.219 \text{ ng/g}
 \end{aligned}$$

% Recovery

$$\% \text{ Recovery} = 100\% \times \frac{(\text{ppb DPX-F6025 found, ng/g})}{(\text{Fortification level, ng/g})}$$

Special Precautions

- A. A column oven (recommend to be set at 35°C) is essential for this method. "Floating" retention times will occur if a column oven is not used.
- B. The bypass tubing [position 2 + 3 (Figure 3)] in the switching valve must be 10 cm, 0.005" Inner Diameter tubing. Longer or wider tubing will result in the analyte missing the time window.
- C. Special attention should be paid to obtaining a very high-quality supply of HPLC-grade acetonitrile for preparation of mobile phases and potassium phosphate (mono- and di-basic) for use in the preparation of buffers. This particular chromatographic separation is very demanding in terms of the solvent purity required, especially for determinations of residues at levels close to the quantitation limit (0.20 ppb). Large system peaks, traced to the presence of solvent impurities, were quite troublesome when using certain lots of acetonitrile during methods development. A reliable brand of acetonitrile is specified in the *Reagents* section.

If possible, a large quantity of acetonitrile from a lot that shows itself to be appropriate should be set aside for this analysis, if much work requiring this residue method is anticipated.

- D. The seals behind the pump heads of the Waters 600 pump are washed daily with 100% Milli-Q® water. This will remove the salt build-up that occurs when the phosphate buffer is used.
- E. All mobile phases are filtered (0.45 µm) prior to use. Buffers and Milli-Q® water were filtered every day prior to analysis. To eliminate bacterial growth in the buffer and water lines, 100% methanol was used to clean out these lines weekly. A 1.0-mL/min flow rate was used and was vented to waste (never went through the column).
- F. Use laboratory techniques consistent with trace organic analysis throughout this procedure.

Cleaning Procedures

All glassware can be cleaned by means of any approach that is consistent with trace organic analysis, such as that used in this study:

Rinse the glass items initially with acetone (technical grade). Follow this with a thorough scrubbing with an aqueous soap solution (prepared in tap water). Then rinse the glassware with tap water, deionized water, followed by another acetone rinse.

HPLC Columns (Preparation for Use)

Appropriate variations of the following procedures are acceptable.

- A. Zorbax® SB-CN (pre-column and Column I) - Pass ≥ 25 mL Milli-Q® water through column (this removes methanol from the column: the column was shipped in methanol and water). Then pass 100-mL mobile phase A through column. Once the retention time for DPX-F6025 stabilizes (mobile phase A through Column I only), this column is ready for use.
- B. Zorbax® ODS - Pass ≥ 25 mL Milli-Q® water through column (the column was shipped in methanol and water), followed by 100-mL mobile phase A. Once the retention time for DPX-F6025 stabilizes (mobile phase A through Column II), this column is ready for use.

Note: None of these HPLC columns should be stored in mobile phase for long periods of disuse (>1-2 weeks). Considerable deterioration of column performance will result if columns are not stored in the solvent in which they were shipped. (Consult manufacturer's literature and use appropriate "intermediate" solvents when making the transition from mobile phase to storage solvent.)

TABLE I
USE OF AN EMPORE™ DISK

Condition

- Pass 20.0 mL of ethyl acetate through the disk.
- Let the disk air dry (under vacuum) for 5 minutes.

Vacuum Settings*

Low Vacuum
 High Vacuum

Pre-Wet

- During these steps, do not let the disk dry out.
- Rinse with 20 mL of methanol
- Rinse with 20 mL of Milli-Q® water
- Rinse with 20 mL of 0.03M phosphate pH 5.0 Buffer

Low Vacuum
 Low Vacuum
 Low Vacuum

Sample Addition

- Add the sample before the disk dries out
- Pass all the sample through the disk
- Let the disk air dry (under vacuum) for 15 minutes

Low Vacuum
 High Vacuum
 High Vacuum

Sample Elution

- Elute DPX-F6025 from the Empore™ disk with three 5-mL portions of ethyl acetate and collect in test tube.

Low Vacuum

Note: With all three 5-mL portions, once some of the 5-mL ethyl acetate has passed through the disk, turn off the pressure and let the disk/ethyl acetate sit for 1-2 minutes. This will increase the yield by 5-15%.

*Low Vacuum: -1.5 inch of Hg

High Vacuum: -20 to -25 inch of Hg

TABLE II
HPLC COLUMN INFORMATION

COLUMN I

Zorbax® SB-CN, 4.6 x 150 mm, 5- μ m particles, #883975-905
(MAC-MOD Analytical, Chadds Ford, PA)

COLUMN II

Zorbax® ODS, 4.6 x 150 mm, 5- μ m particles, #883952-702
(MAC-MOD Analytical, Inc., Chadds Ford, PA)

or

Zorbax® ODS, 4.6 x 250 mm, 5- μ m particles, #880952-702
(MAC-MOD Analytical, Inc., Chadds Ford, PA)

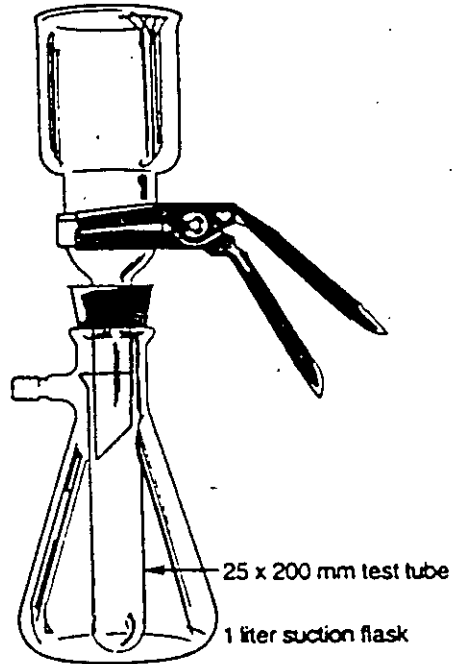
TABLE III
TIMING SEQUENCE FOR CHLORIMURON ETHYL (COLSW2 PROGRAM)

Program	Time min	Flow Rate mL/min	% A Buffer	% B ACN	% C Water	% D MeOH	Column	Action
CS-START	0	1.5	55	45	0	0	I + II	Equilibrate Column II
	10	1.0	55	45	0	0	I + II	Equilibrate Column II
	15	2.0	65	35	0	0	I	Equilibrate Column I
	25	1.0	65	35	0	0	I	Equilibrate Column I
	30							Program end
CS1	0	1.0	65	35	0	0	I	Start of program Injection occurred
	RT -0.2	1.0	65	35	0	0	I + II	Column switched to Column II
	RT +0.2	1.0	65	35	0	0	I	Column switched back to Column I
	20	2.0	20	80	0	0	I	Cleaning Column I
	26	3.0	55	45	0	0	I	Equilibration of Column I
	36	1.0	55	45	0	0	I	Equilibration of Column I
	41	1.0	55	45	0	0	I + II	Analytical Separation on Column II
	66	3.0	65	35	0	0	I	Equilibration of Column I
	71	1.0	65	35	0	0	I	Equilibration of Column I
	75							Program end
	FINAL	0	1	0	0	100	0	I + II
5		2	0	0	100	0	I + II	
30		1	0	0	100	0	I + II	
40		2	0	0	0	100	I + II	Condition Columns for Storage
50		1	0	0	0	100	I + II	
59.99		0	0	0	0	0	I + II	Spurge & Flow Turned Off
60								Program End

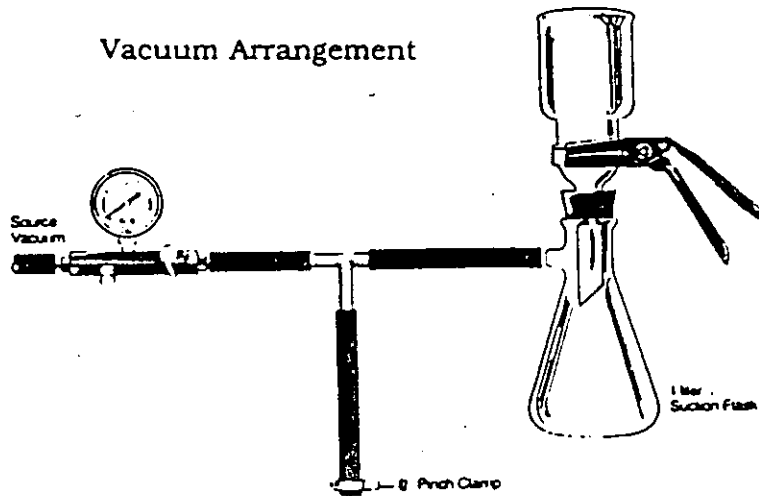
TABLE IV
THE RETENTION TIME FIND PROGRAM (COLSW1 PROGRAM)

Program	Time min	Flow Rate mL/min	% A Buffer	% B ACN	%C Water	%D MeOH	Column	Action
RT- START	0	2	65	35	0	0	I	Equilibrate Column I
	15	1.0	65	35	0	0	I	Equilibrate Column I
	30							Program End
RTFIND	0	1.0	65	35	0	0	I	Start of program Injection occurred
	20							Program End
FINAL	0	1	0	0	100	0	I + II	Remove Buffer
	5	2	0	0	100	0	I + II	
	30	1	0	0	100	0	I + II	
	40	2	0	0	0	100	I + II	Condition Columns for Storage
	50	1	0	0	0	100	I + II	
	59.99	0	0	0	0	0	I + II	Spurge & Flow Turned Off
	60							Program End

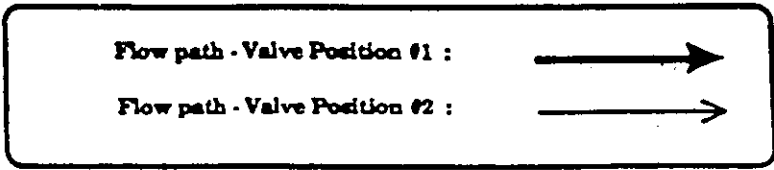
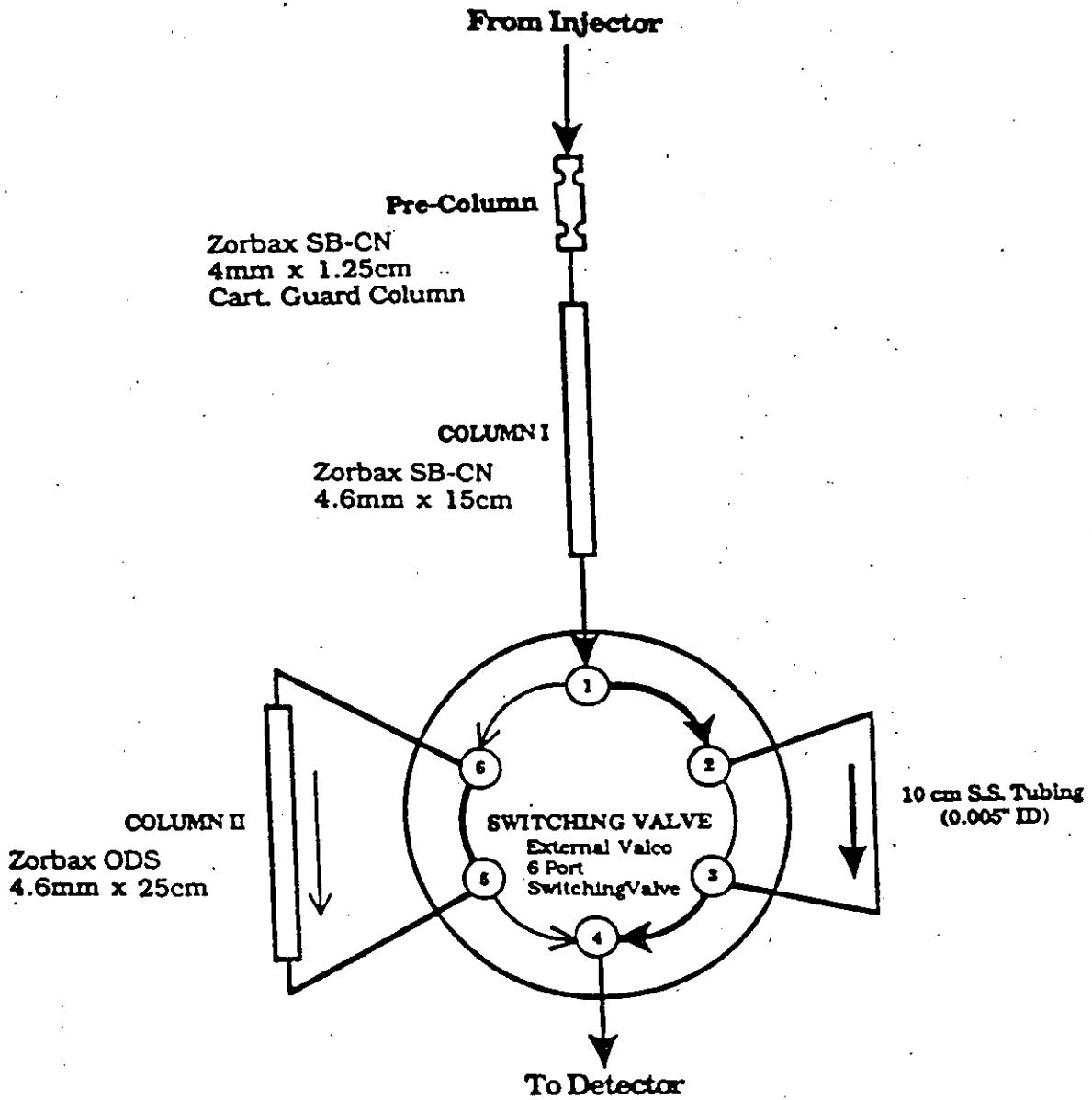
FIGURE 1
EMPORE™ ELUTION APPARATUS AND VACUUM ARRANGEMENT



Elution Apparatus



**FIGURE 3
PLUMBING DIAGRAM FOR COLUMN SWITCHING**



All tubing used is Stainless Steel 0.01"ID unless stated otherwise

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