Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Pyridate

MRID #: 422968-01

Matrix: Soil

Analysis: HPLC/UV

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EN-CAS METHOD NO. ENC-16/90

Analytical Procedure for the Determination of Pyridate and Its Primary Metabolites CL-9673 and CL-9673-O-Methyl in Soil

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1.0 INTRODUCTION

1.1 Scope

This method is used for the determination of pyridate and its primary metabolites CL-9673 and CL-9673-O-Methyl in soil. The method has been successfully applied to soils from North Carolina and has also been shown to work with various soil types from other locations. Concentrations as low as 0.02 ppm of each analyte can be determined. The method has also been validated on residues as high as 0.40 ppm and has been shown by subsequent analysis to be applicable to residue concentrations of at least 1.0 ppm. Method validation results from EN-CAS report 90-0075, Pyridate - Terrestrial Field Dissipation - Peanuts - NC, are included in this method (see Tables I to IV). See Figure 1 for a flowchart of the method.

1.2 Principle

Pyridate and its primary metabolites are extracted from soil by shaking in methanol (MeOH). The MeOH extract is acidified with acetic acid to minimize conversion of pyridate to CL-9673 during subsequent method steps and during storage. Aliquots equivalent to 10 g of soil are reduced to dryness and the analytes are selectively transferred with organic and aqueous washes. Following acidification of the aqueous wash, the organic and aqueous phases are partitioned in 90:10 hexane:dichloromethane (DCM) in order to completely segregate the organic soluble components (pyridate and CL-9673-O-Methyl) from the aqueous soluble component (CL-9673). The aqueous fraction is partitioned a second time with 90:10 hexane:DCM to transfer any residual

1.2 Principle (continued)

CL-9673-O-Methyl remaining in the aqueous phase to the organic phase. Following separation of the phases, an appropriate volume of HPLC-grade MeOH is added to the aqueous phase and the sample is analyzed by High Performance Liquid Chromatography (HPLC). The organic phase containing the residual CL-9673-O-Methyl is combined with the earlier organic wash so that virtually all of the extracted pyridate and the CL-9673-O-Methyl metabolite are contained in a single organic fraction. For HPLC analysis, pyridate in the organic phase is converted to CL-9673 using morpholine. The organic phase is reduced to dryness and the residue is reconstituted and brought to an appropriate final volume with ammonium acetate buffer/MeOH. The pH is adjusted to 5 and the sample is analyzed by HPLC. The HPLC system used for these analyses utilizes double-column switching to direct a "heart-cut" from a 60 mm x 4.6 mm C_{18} column to a second, 60 mm x 4.6 mm C_{18} column. Longer columns (75 mm x 4.6 mm C_{18}) may also be used as appropriate for the analyses.

Pyridate (as CL-9673) and CL-9673-O-Methyl, from the organic fraction, are co-analyzed by UV detection at 280 nm and 254 nm respectively. Free CL-9673, derived from the aqueous fraction, is separately injected and quantitated at 280 nm. This method is capable of determining residues to 0.02 ppm of each component.

2.0 APPARATUS

Note: All equipment/apparatus may be replaced by equivalent items from alternate sources.

2.1 Bottles, 16 oz, French square, amber wide mouth with Teflon-lined caps

2.2 Funnels, Buchner, 9 cm

2.3 Flasks, vacuum, 500 ml

2.4 Graduated cylinders, 100 ml, and 500 ml

2.5 Flasks, Erlenmeyer, 500 ml

2.0 APPARATUS (continued)

2.6 Flasks, 250 ml, flat-bottom with ground glass joint, silanized

Note: The silanizing solution is made with 95:5 hexane:dimethyldichlorosilane. Before silanizing, all glassware must be clean and dry. Silanizing must take place under a hood, with the proper protective clothing. The glassware is rinsed (coating with a thin layer) with silanizing solution and allowed to dry overnight or several hours in a hood. The glassware is then rinsed thoroughly with D.I. water, followed by acetone, and then dried on a rack.

2.7 Tubes, centrifuge, graduated and ungraduated, 15 ml with Teflon-lined caps

2.8 Tubes, for Turbovap Evaporator, 15 ml with plastic snap caps, Zymark Inc.

2.9 HPLC vials, 4 ml with Teflon lined caps

2.10 Pipets, disposable, 2 ml

2.11 Stoppers, 24/40, polyethylene

2.12 Pipets volumetric, (various sizes)

2.13 Flasks, volumetric, 100 ml, 250 ml, and 500 ml

2.14 Glass fiber filter peper, Whatman 934-AH, 9.0 cm and 12.5 cm

2.15 Vacuum manifold apparatus for filtering

2.16 Pipettes, Eppendorf, 10-100 µl (w/tips), and Oxford Macro Set, 1-5 ml (w/tips)

2.17 Syringes, 100 µl, 500 µl, 1000 µl (Hamilton)

2.18 pH sticks in ranges: 0-6, 0-14, 5-10, and 7.5-14

2.19 Syringe filters, Anotop 25, 0.2 µm, 45 mm

2.20 Polypropylene sheets

3.0 EQUIPMENT

3.1 Laboratory mechanical shaker, G10 Gyrotory, New Brunswick Scientific Co., Inc.

3.2 Rotary evaporator, Buchi Rotovapor, model #RE111

3.3 Ultrasonic bath, Branson 5200

3.4 Small vortexer, with pulse mode, Glas-Col

3.5 Centrifuge, 24 port, Fisher Scientific, model 225

3.6 pH meter, Accumet 925, Fisher Scientific

3.7 Turbovap evaporator, Zymark LV model

3.8 Nitrogen evaporator, Organomation N-Evap model 112

3.9 Analytical balance, Mettler, capable of 0.00001 g accuracy, ± 0.01 mg for weighing analytical standards

3.10 Top loading balance, American Scientific Products, TL160G, ± 0.01 g accuracy

4.0 REAGENTS

4.1 Methanol (MeOH), pesticide and HPLC grades

4.2 Dichloromethane (DCM), pesticide grade

4.3 Hexane, pesticide and HPLC grades

4.4 Water, HPLC grade

4.5 Acetic acid, A.C.S. reagent grade

4.6 Acetic acid, HPLC grade

4.7 Ammonium Hydroxide (>25%)

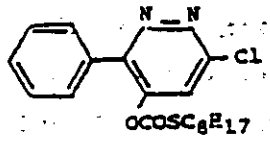
4.8 Morpholine, 99+%

4.9 Ammonium Acetate

4.10 95:5 HPLC hexane:dimethyldichlorosilane (silanizing solution)

5.0 TEST SUBSTANCES

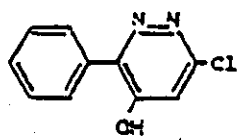
5.1 Pyridate Structure, Chemical and Physical Characteristics



$C_{19}H_{23}ClN_2O_2S$

Chemical Name:	O-(6-chloro-3-phenyl-4-pyridazinyl)-S-octyl-carbonothioate
Molecular Weight:	379
Description:	Clear brown liquid at room temperature with a mercaptan like odor
Melting Point:	27°C (pure substance)
Solubility in Water:	1.5 mg/L at 20°C
Thermal Decomposition:	Begins at 30°C
Storage Conditions:	Freezer at -10°C to -17°C
Storage Stability:	At least 1 year
Purity:	98%

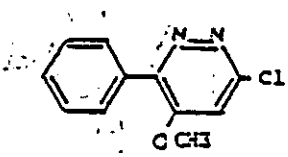
5.2 CL-9673 Structure, Chemical and Physical Characteristics



$C_{10}H_7ClN_2O$

Chemical Name:	3-phenyl-6-chloro-pyridazinol-4
Molecular Weight:	206.5
Description:	White odorless crystals
Melting Point:	225°C
Solubility in Water:	37 mg/L at 20°C
Thermal Decomposition:	>225°C
Storage Conditions:	Freezer at -10°C to -17°C
Storage Stability:	At least one year
Purity:	97.2%

5.3 CL-9673-O-Methyl Structure, Chemical and Physical Characteristics



$C_{11}H_9ClN_2O$

Chemical Name:	3-phenyl-4-methoxy-6-chloropyridazine
Molecular Weight:	220
Description:	White odorless crystals
Melting Point:	127.5°C
Solubility in Water:	0.15 g/L
Thermal Decomposition:	No spontaneous decomposition up to 100°C
Storage Conditions:	Freezer at -10°C to -17°C
Storage Stability:	At least 1 year
Purity:	99.6%

6.0 STANDARD STOCKS AND ANALYTICAL SOLUTIONS

6.1 Pyridate Standards

In 100 ml of hexane, dissolve an exact weight of pyridate to produce a stock concentration of 0.50 mg/ml. Serial dilutions from the stock standard may be made to appropriate concentrations for fortification standards. There are no calibration standards for pyridate since pyridate is quantitated as CL-9673. The stock standard and fortification standards are stable for 3 months. Store all standards in the freezer at a temperature of -10°C to -17°C, protected from light.

6.2 CL-9673 Standards

In 100 ml of HPLC MeOH, dissolve an exact weight of standard to give a stock concentration of 0.50 mg/ml. Serial dilutions from the stock standard may be made to appropriate concentrations for fortification standards and calibration standards (calibration standards are prepared with pH 9 ammonium acetate buffer*/MeOH, 100/5 ppv). Adjust the pH to 5 with acetic acid. Typical CL-9673 calibration standards range from 0.0125 µg/ml to 1.0 µg/ml. The stock standard, fortification standards, and calibration standards are stable for 6 months. Store all standards in the freezer at a temperature of -10°C to -17°C, protected from light.

6.3 CL-9673-O-Methyl Standards

In 100 ml of HPLC grade MeOH, dissolve an exact weight of standard to give a stock concentration of 0.50 mg/ml. Serial dilutions from the stock standard may be made to appropriate concentrations for fortification standards and calibration standards [calibration standards are prepared with pH 9 ammonium acetate buffer*/MeOH, 100/5 parts per volume (ppv)]. Typical CL-9673-O-methyl calibration standards range from 0.025 µg/ml to 1.0 µg/ml. The stock standard, fortification standards, and calibration standards are stable for 6 months. Store all standards in the freezer at a temperature of -10°C to -17°C, protected from light.

* See section 7.4.

7.0 PREPARATION OF REAGENT AND MOBILE PHASE SOLUTIONS

7.1 Ammonium Acetate Buffer

Weigh 15.4 g of anhydrous ammonium acetate and dissolve in deionized water in a 1000 ml volumetric flask. Bring to volume with deionized water and adjust pH up to 9.0 with ammonium hydroxide using a pH meter.

7.2 pH Adjusted Buffer (Aqueous Transfer Solvent)

Adjust the pH of the ammonium acetate buffer (pH 9) to a pH of 8.0 with acetic acid for residue transfer partition (see Section 8.3).

7.3 Hexane/Dichloromethane (Organic Extraction Solvent)

Prepare a 90:10 solution of hexane and dichloromethane (DCM).

7.4 Final Volume Solvent

Prepare 100 ml of ammonium acetate (pH 9) using a 100 ml volumetric flask. After the correct volume (100 ml) is reached, pipet 5 ml of MeOH into the volumetric flask and mix well.

7.5 Mobile Phase 1 (Solvent A plus Solvent B)

Solvent A - Add 20 ml of acetic acid (Baker) to 4000 ml HPLC grade MeOH. Filter mixture through a 0.45 µm filter. Degas by bubbling high purity helium through a dispersion frit at 100 ml/min. for a minimum of 2 to 3 hours before use.

Solvent B - Add 20 ml of acetic acid to 4000 ml HPLC grade water. Filter through a 0.45 µm filter. Degas as described for solvent A for a minimum of 3 hours before use.

7.6 Mobile Phase 2 (Column 1 Flush)

Add 20 ml of acetic acid to 4000 ml HPLC grade MeOH. Filter through a 0.45 µm filter. Degas as described in Section 7.5.

7.6 Mobile Phase 2 (Column 1 Flush) (continued)

NOTE: Following HPLC system equilibration, decrease helium flow to 50 ml/min. to prevent compositional changes due to selective evaporation of the more volatile solvent components.

NOTE: The gradient composition of the mobile phase may need to be varied depending upon operational requirements for chromatographic separation.

8.0 ANALYTICAL PROCEDURE

See Figure 1 for a flowchart of the method.

8.1 Sample Preparation

Sift the soil sample through a 2 mm screen. Separate a representative subsample for use in performing the analysis. If the sample cannot be analyzed immediately, store in a freezer at -23°C to -27°C. The moisture content of the soil sample is determined by a weight-by-difference method as outlined by EN-CAS SOP III-5.3.

8.2 Extraction

Weigh a 50 g representative soil sample into a 16 oz. amber wide-mouth French square bottle and add 150 ml of MeOH. Cover the mouth of the bottle with a sheet of polyethylene, cap tightly with a Teflon lined cap and place the bottle on its side on a mechanical shaker. Shake at 200 rpm for 15 minutes. Decant the extract into a 9 cm Buchner funnel containing a Whatman GF/C 12.5 cm filter on top of a Whatman GF/C 9 cm filter. Vacuum filter the sample into a 500 ml sidearm flask at a vacuum of 5-15 mm Hg. Repeat the extraction two more times using 150 ml of MeOH each time, combining the collected fractions. Adjust the final total volume to 500 ml with MeOH. Add 2.5 ml (0.5%) of acetic acid to serve as a stabilizer for pyridate. Transfer a 10 g aliquot (100 ml) of the extract into a well silanized (see Section 2.6 for description of silanizing solution) 250 ml flat-bottom flask.

8.2 Extraction (continued)

Concentrate to dryness on a rotary evaporator with a water bath at 40°C. Pour the remaining extract into an appropriate bottle and store under standard freezer temperatures.

Note: Successful reanalysis of the sample can be achieved by taking an additional aliquot from stored extracts that have been stored up to 14 days.

8.3 Sample Transfer

Add 4 ml of 90:10 hexane:DCM to the evaporation flask and place in a ultrasonic bath for 30 seconds while simultaneously rotating the flask so that all of the flask walls are well rinsed. Transfer the 90:10 wash into a 15 ml centrifuge tube labeled (1). Add a second 2 ml portion of 90:10 hexane:DCM to the residue flask and sonicate for 30 seconds. Transfer the second wash to tube (1). [The 90:10 washes should contain mainly the pyridate and CL-9673-O-methyl compounds.]

Using a gentle stream of nitrogen gas (approximate flow rate 0.4 L/min.), evaporate any residual 90:10 hexane:DCM from the residue flask. Volumetrically add 4 ml of ammonium acetate buffer (pH 8) to the residue flask and sonicate for one minute rotating the flask as before. Transfer the buffer wash into a second 15 ml centrifuge tube labeled (2). Volumetrically add a second 2 ml buffer wash to the residue flask. Sonicate one minute and transfer to tube (2). Acidify the 6 ml of buffer in tube (2) to a pH of 5.0 with acetic acid, and allow to stand for a minimum of 30-45 minutes. The aqueous buffer in tube (2) should contain mainly CL-9673.

8.4 Partition

This partition is performed to ensure complete separation of pyridate and CL-9673-O-Methyl into the organic phase and CL-9673 into the aqueous phase.

Transfer the 90:10 hexane:DCM from tube (1) to tube (2) containing the acidified buffer. Vortex tube (2) for 5 minutes then centrifuge for 5 minutes at 2000 rpm.

8.4 Partition (continued)

Using a disposable pipet, transfer the organic (top) layer from tube (2) back to tube (1), being careful not to remove any aqueous from tube (2). A small amount of 90:10 hexane:DCM should be left in tube (2) to be certain that no aqueous is removed. Add an additional 6 ml of 90:10 hexane:DCM to the residue evaporation flask as a final rinse. Sonicate for 30 seconds and transfer to tube (2) for a second partition of the aqueous phase. Vortex tube (2) for 5 minutes then centrifuge for 5 minutes. Transfer the organic (top) layer from tube (2) to tube (1), again leaving behind a small quantity of 90:10 hexane:DCM to ensure that no aqueous is transferred. Add an additional 1 ml portion of 90:10 hexane:DCM directly into tube (2), and gently swirl. Transfer the majority of the 90:10 hexane:DCM to tube (1) being careful not to transfer any aqueous from tube (2).

8.5 Aqueous (CL-9673) Sample Preparation

Evaporate residual hexane:DCM from the surface of the buffer layer in tube (2) using a gentle stream of nitrogen. Add 300 µl of HPLC grade methanol and record the final volume. Pass sample through a 0.2 µm Anotop 25 mm syringe filter into a 4 ml glass auto-injection vial for HPLC analysis.

8.6 Organic (Parent and CL-9673-O-Methyl) Sample Preparation

Add 50 µl of morpholine to the combined organic (90:10 hexane:DCM) fractions in tube (1) and mix well. [Morpholine rapidly converts pyridate to CL-9673]. Concentrate the contents in tube (1) to dryness using a stream of nitrogen gas (flow rate of 5 PSI gradually increasing to 20 PSI) using a Zymark Turbovap LV with a bath temperature of 30°C. Reconstitute the sample with 4 mls of 100/5 ppv ammonium acetate buffer pH 9/MeOH, and sonicate for 10 minutes. Transfer the sample to a 4 ml HPLC glass auto-injection vial and adjust the pH to 5 with acetic acid (this should take 20-30 µl of acetic acid, but should not exceed 50 µl).

8.7 Time Required for Analysis

A skilled analyst should be able to complete the sample preparation for a set of 5 samples including control, fortified samples, and reagent blanks in approximately 1.0 day. HPLC analysis can be achieved overnight via an automated sampling system.

8.8 Detection Limit

This method permits a limit of quantitation (LOQ) in soil of 0.02 ppm each for pyridate (determined as CL-9673), CL-9673, and CL-9673-O-Methyl. Adjust instrument sensitivity, analytical standards and sample volumes to allow detection of each analyte to 50% of the LOQ.

8.9 Safety Precautions

Normal safety precautions, including the wearing of gloves and safety glasses, and the use of a fume hood, are recommended to minimize exposure to the analyte and organic solvents used in this procedure.

9.0 COMMENTS

Experimental evidence indicates that a small amount (≤5%) of CL-9673 may be observed in the organic phase of samples fortified with CL-9673 only. The main cause of this phenomenon is the tendency for a small percentage (2-3%) of CL-9673 to partition into hexane. When dichloromethane is added (i.e. 90:10 hexane:DCM) this percentage increases slightly.

The appearance of CL-9673 in the organic phase may also be enhanced in certain soils where the CL-9673 "complexes" into an organic-soluble form. This prevents optimum partitioning of the CL-9673 into the aqueous phase. Acidification of the aqueous phase (see section 8.3) releases CL-9673 from this "complex" so that virtually all of the CL-9673 remains in the aqueous phase. Sample chromatograms illustrating this phenomenon can be found in Figures 20, 21, and 28.

Significant degradation of pyridate to the CL-9673 metabolite has been observed during rotary evaporation of the MeOH extract. This degradation can be greatly reduced by the addition of a small percentage (0.5%) of acetic acid to the extract prior to evaporation. The

9.0 COMMENTS (continued)

acid also appears to prolong the storage life of pyridate in extracts from soil. Variations in the amount of acetic acid needed may be necessary based on different soil types.

10.0 HPLC INSTRUMENT DESCRIPTIONS, TECHNIQUES, AND OPERATING CONDITIONS

Sample injections are loaded onto column 1 using mobile phase 1. The portion of the eluent from column 1 containing the analytes is directed by a time programmed valve switching system to column 2 where further separation occurs. During this period, column 1 is flushed with mobile phase 2 (see Figures 2-6).

Instrument:	Multisolvent gradient delivery system (Waters model 600E)
Detector:	UV (Waters 490E) Xenon lamp Sensitivity at 0.500 AUFS
	UV Settings
	Pyridate as CL-9673 - 280 nm CL-9673-O-Methyl - 254 nm CL-9673 - 280 nm
Injector:	Auto/Programmable (Waters, WISP 712)
	Injection Volume: 75 µl-300 µl
Pump #1:	Gradient 600E, 1000-2000 PSI
Mobile Phase #1:	MeOH/Acetic acid 1000/5
	20-40% Solvent A A = 1000/5 ppv* MeOH/acetic acid
	50-80% Solvent B B = 1000/5 ppv water/acetic acid
	Flow Rate: 1.0 ml/min. (pump 1 & 2)

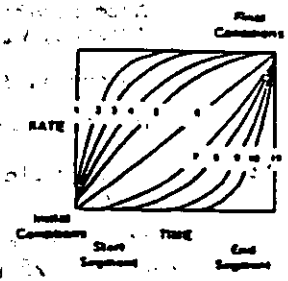
* Parts per volume.

10.0 HPLC INSTRUMENT DESCRIPTIONS, TECHNIQUES, AND OPERATING CONDITIONS (continued)

MOBILE PHASE #1 GRADIENT TABLE

TIME (min.)	FLOW (ml/min.)	% A	% B	CURVE
INITIAL	1.0	47.5-50	52.5-50	-
3.5-4.0	1.0	20 - 25	80 - 75	11
6.0	1.0	45	55	11
9.0-20	1.0	47.5-50	52.5-50	11
30.0	1.0	47.5-50	52.5-50	11
40.0	0.1	47.5-50	52.5-50	6

WATERS 600E GRADIENT CURVES



NOTE: Ranges given for both gradient table and valve switching reflect instrument/column variations.

NOTE: The gradient composition of the mobile phase may need to be varied depending upon operational requirements for chromatographic separation.

10.0 HPLC INSTRUMENT DESCRIPTIONS, TECHNIQUES, AND OPERATING CONDITIONS (continued)

Pump #2	Isocratic (Waters model 510), 500 PSI
Mobile Phase #2:	MeOH/Acetic acid 1000/5 Flow Rate: 1.0 ml/min. (pump 1 & 2)
Column Oven Temperature:	30° - 40°C
Columns:	Bonded Phase: C_{18} Nucleosil 100Å, 5µm (Alltech) Dimensions: 60 mm X 4.6 mm OR Bonded Phase: C_{18} Nucleosil 100Å, 5µm (Keystone) Dimensions: 75 mm X 4.6 mm
Valves:	2, 6-Port programmable, Rheodyne pneumatic or Valco electronic
Approximate Retention Times:	Column 1 CL-9673 - 1.6 min. CL-9673-O-Methyl - 2.1 min. Column 1 and 2 CL-9673 - 7.8 min. CL-9673-O-Methyl - 13.0 min.

10.0 HPLC INSTRUMENT DESCRIPTIONS, TECHNIQUES, AND OPERATING CONDITIONS (continued)

Integrator Parameters: Hewlett-Packard 3396A Integrator

Parameter Definitions	Run Parameters	Timetable Events
0. SET BASELINE MODE	ZERO = 20	0.000 CHT SP = 0.5
1. SET BASELINE NEXT VALLEY	ATT 2^ = 5	0.000 THRG / = 3
2. SET BASELINE ALL VALLEYS	CHT SP = 0.5	0.000 THRG / = 2
3. SKIM FROM NEXT PEAK	AR REJ = 0	11.000 STOP
4. DISABLE AUTO-TANGENT SKIMMING	THRSH = -1	
5 EXTEND BASELINE HORIZONTALLY	PK WD = 0.35	
6. MEASURE AND UPDATE THRESHOLD		
7. TURN OFF RETENTION TIME LABELING		
8. TURN ON START/STOP BIAS		
9. TURN OFF INTEGRATION		
10. INCREMENT THRESHOLD		
11. INVERT NEGATIVE PEAKS		
12. CLAMP NEGATIVE PEAKS		
13. SHOW EV11, EV12		
14. START PEAK SUM WINDOW		

10.1 Heart-Cut Procedure

Set the detector wavelength to 280 nm and program the other instrumental parameters as listed under HPLC Instrument Descriptions, Techniques, and Operating Conditions in Section 10.0. Determine the retention time of a 0.25 µg/ml CL-9673 standard on column 1 by connecting column 1 directly to the detector. Program the valves to permit elution of column 1 with mobile phase 1 (see Section 10.3). Identify and note the CL-9673 retention time.

Next, inject a 0.25 µg/ml CL-9673-O-Methyl standard under the same conditions outlined for CL-9673, except change the detector wavelength to 254 nm. Identify and note the CL-9673-O-Methyl retention time. [IMPORTANT: Make sure the peaks are as close to full scale as possible in both instances to permit an accurate measurement of the peak width.]

10.0 HPLC INSTRUMENT DESCRIPTIONS, TECHNIQUES, AND OPERATING CONDITIONS (continued)

Determine the heart-cut interval for CL-9673 plus CL-9673-O-Methyl by measuring the analyte peak width at one-half of the peak height and multiplying by a factor of 1.5. Convert this number to centimeters and divide by the chart speed to obtain the time required for the analyte to elute. Subtract the time value obtained with the CL-9673 standard from the CL-9673 retention time to establish the onset of the heart-cut. Termination of the heart-cut occurs at the retention time of the CL-9673-O-methyl standard plus the calculated heart-cut value in minutes. See Figures 8 and 9 for sample chromatograms showing column profiles and the calculation of the heart-cut. Reconnect column 1 to valve 1 and return the feed-in line from valve 2 to the inlet port on the detector.

10.2 Standardization

Calibrate the HPLC system periodically or when problems arise with drifting retention times by comparing the retention times (±2%) within each run and/or (±5%) with previous runs.

Standardize the system by injecting a series of CL-9673 injection standards (i.e. 0.025 µg/ml to 0.5 µg/ml). Construct a calibration curve from the data by linear regression.

10.3 Valve switching

Two types of valves have been used with this system. Rheodyne (Waters) pneumatically controlled actuators and Valco (Alltech) electronically controlled actuators. Figure 2-4 shows the schematic representation of the Rheodyne valves (see Table V for the timing events of these valves). Figure 5-7 and Table VI outline the same type of information for the Valco actuators.

10.0 HPLC INSTRUMENT DESCRIPTIONS, TECHNIQUES, AND OPERATING CONDITIONS (continued)

10.4 Representative Chromatograms

Typical chromatograms of the organic phase represent analysis of pyridate as CL-9673 and the CL-9673-O-Methyl metabolite (see Figures 14-16 for the 0-12" depth, and Figures 26-28 for the 12-24" depth). Analysis of CL-9673 is represented in the chromatograms of the aqueous phase (see Figures 17-19 for the 0-12" depth, and Figures 29 and 30 for the 12-24" depth). Chromatograms from the 24-36" layer are very similar to those from the 12-24" layer and therefore are not included in the report.

In addition, several chromatograms representing complementary (organic) fractions of samples fortified with CL-9673 are included to show that at higher concentrations of analyte (i.e. $\geq$ 0.20 ppm), small amounts of CL-9673 can be found in the organic phase. This phenomenon was discussed in the comments section (Section 9.0) of this report.

11.0 CALCULATIONS

11.1 Calculation of ng Injected

$$ng\ inj. = \frac{\text{sample wt. (g)} \times \text{aliquot (ml)} \times \mu l\ \text{injected} \times 1000\ ng/g}{[\text{ml total extract volume} \div (g\ \text{sample} \times \text{decimal \% } H_2O)] \times \mu l\ F.V. \times V_{af}/V_i}$$

F.V. = Final volume
V_i = Initial volume diluted
V_{af} = Adjusted final volume
V_{af}/V_i = Dilution factor

11.0 CALCULATIONS (continued)

11.2 Calculation of Net ppm Residue

ng found is determined from a standard curve using the equation:

$$\text{ng found} = \frac{\text{peak height} - y \text{ intercept}}{\text{slope}}$$

$$\text{ppm (wet)} = \frac{\text{ng found in injected sample}}{\text{ng injected}}$$

11.3 Calculation for Moisture Correction and Molecular Weight Conversion Factor (if applicable)

$$\text{ppm (dry weight basis)} = \frac{\text{ppm (wet weight basis)}}{1 - \text{decimal \% } H_2O}$$

Molecular weight conversion factor for determination of pyridate from CL-9673:

$$\frac{\text{Pyridate}}{\text{CL-9673}} = \frac{379}{206.5} = 1.83$$

Corrected ppm = ppm (dry weight basis) x MW Factor

11.4 Calculation of Procedural Recovery (R%)

$$R\% = \frac{(\text{ppm wet} - \text{ppm wet control})\ (\text{MW factor})^{*}}{\text{fortification level (ppm)}}$$

* Molecular weight conversion factor of 1.83 is used for pyridate only.

11.0 CALCULATIONS (continued)

11.5 Example Calculation

$$\text{ng injected} = \frac{50\ \text{g} \times 100\ \text{ml} \times 250\ \mu\text{l} \times 1000\ \text{ng/g}}{[500\ \text{ml} + (50\ \text{g} \times 0.09)] \times 4000\ \mu\text{l} \times 1} = 619.43\ \text{ng}$$

$$\text{ng found} = \frac{204043 - (-1689.31)}{1458.749} = 139.7\ \text{ng}$$

$$\text{ppm (wet)} = \frac{139.7\ \text{ng found}}{619.43\ \text{ng injected}} = 0.2255\ \text{ppm}$$

$$\text{ppm (dry)} = \frac{0.2255\ \text{ppm}}{1 - 0.09} = 0.2478\ \text{ppm}$$

ppm corrected for % moisture = 0.2478 ppm x MW Factor*

To calculate % Recovery (% R):

$$\% R = \frac{(0.2255\ \text{ppm} - 0) \times \text{MW Factor}^*}{0.25\ \text{ppm}} \times 100$$

$$= 0.90$$

* Molecular weight conversion factor of 1.43 is used for pyridate only.

12.0 VALIDATION RESULTS

Validation data is presented in Tables I - IV. Results of this data, including recovery mean and standard deviations, establish reliability of the method for the determination of pyridate and its primary metabolites. Also note the occasional residues of free CL-9673 in the complementary organic fractions as discussed in the comments section 7.9.1 of the method.

12.0 VALIDATION RESULTS

12.1 North Carolina - Peanut Soil

The North Carolina validation involved analysis of 30 samples at three fortification levels (0.04, 0.20 and 0.40 ppm) comprising soil depths from 0-36". For each analyte there were 6 samples fortified at 0.04 ppm, 2 at 0.20 ppm, and 2 at 0.40 ppm. The mean and standard deviation for pyridate analyses is 84.7% ± 5.7 (n=10), 85.9% ± 5.9 (n=10) for CL-9673-O-Methyl analyses, and 84.8% ± 6.7 (n=10) for CL-9673 analyses.

12.2 Additional Validation Data

Additional validation data from other sites may be added by addendum to this method.

13.0 REFERENCES

13.1 Agrolinz Agrarchemikalien GmbH Report No. 1064, Method of Analysis for Determination of Pyridate and its Main Metabolites CL-9673 and CL-9673-O-Methyl in Soil, issued August 1990.

Table I

Pyridate Method Validation Results

EN-CAS Sample #	EN-CAS Analysis Set # and Soil Depth	Fortification Level (ppm)	Pyridate ppm Found or % Recovered: Organic Phase [1]		Aqueous Phase
EI9104-C1	NV/0-12"	-	<0.02	-	(<0.02)
EI9104-C2	NV/0-12"	-	<0.02	-	(<0.02)
EI9104-S1	NV/0-12"	0.04	0.0366	92	
EI9104-S2	NV/0-12"	0.04	0.0364	91	
EI9104-S3	NV/0-12"	0.20	0.1751	88	
EI9104-S4	NV/0-12"	0.20	0.1684	84	
EI9104-S5 a	NV/0-12"	0.40	0.3404	85	
EI9104-S6	NV/0-12"	0.40	0.3519	88	
RBLK b	NV/0-12"	-	<0.02	-	(<0.02)
EI9106-C1	NV/12-24"	-	<0.02	-	(<0.02)
EI9106-C2	NV/12-24"	-	<0.02	-	(<0.02)
EI9106-S1	NV/12-24"	0.04	0.0328	82	(<0.02)
EI9106-S2	NV/12-24"	0.04	0.0306	76	(<0.02)
EI9108-C	NV/24-36"	-	<0.02	-	(<0.02)
EI9108-C2	NV/24-36"	-	<0.02	-	(<0.02)
EI9108-S1	NV/24-36"	0.04	0.0346	86	
EI9108-S2	NV/24-36"	0.04	0.0300	75	
			Mean =	84.7%	
			Standard Deviation =	± 5.7	
			(n=10)		

a Realiquot: The original sample yielded low recovery (%) due to in-lab sample loss.
b Reagent blank.
( ) Parenthetical information represents complementary fraction.

Table II

Pyridate Method Validation Results

EN-CAS Sample #	EN-CAS Analysis Set # and Soil Depth	Fortification Level (ppm)	CL-9673-O-Methyl ppm Found or % Recovered: Organic Phase		Aqueous Phase
EN9104-C1	NY/0-12"	-	<0.02	-	(<0.02)
EN9104-C2	NY/0-12"	-	<0.02	-	(<0.02)
EN9104-S1	NY/0-12"	0.04	0.0342	85	
EN9104-S2	NY/0-12"	0.04	0.0370	93	
EN9104-S3	NY/0-12"	0.20	0.1815	91	
EN9104-S4	NY/0-12"	0.20	0.1561	78	
EN9104-S5 a	NY/0-12"	0.40	0.3468	87	
EN9104-S6	NY/0-12"	0.40	0.3231	81	
MBLK b	NY/0-12"	-	<0.02	-	(<0.02)
EN9106-C1	NY/12-24"	-	<0.02	-	(<0.02)
EN9106-C2	NY/12-24"	-	<0.02	-	(<0.02)
EN9106-S1	NY/12-24"	0.04	0.0367	92	(<0.02)
EN9106-S2	NY/12-24"	0.04	0.0358	89	(<0.02)
EN9108-C	NY/24-36"	-	<0.02	-	(<0.02)
EN9108-C2	NY/24-36"	-	<0.02	-	(<0.02)
EN9108-S1	NY/24-36"	0.04	0.0349	87	
EN9108-S2	NY/24-36"	0.04	0.0303	76	
			Mean =	85.9%	
			Standard Deviation =	± 5.9	
			(n=10)		

a Analiquot: The original sample yielded low recovery (%) due to in-lab sample loss.
b Reagent blank.
( ) Parenthetical information represents complementary fraction.

Table III

Pyridate Method Validation Results

EN-CAS Sample #	EN-CAS Analysis Set # and Soil Depth	Fortification Level (ppm)	CL-9673 ppm Found or % Recovered: Aqueous Phase		CL-9673 ppm Found or % Recovered: Organic Phase
EI9104-C1	NV/0-12"	-	<0.02 a	-	<0.02
EI9104-C2	NV/0-12"	-	<0.02 a	-	<0.02
EI9104-S7	NV/0-12"	0.04	0.0371	85	
EI9104-S8	NV/0-12"	0.04	0.0326	73	
EI9104-S9	NV/0-12"	0.20	0.1680	82	
EI9104-S10	NV/0-12"	0.20	0.1670	82	
EI9104-S11 b	NV/0-12"	0.40	0.1216	80	
EI9104-S12	NV/0-12"	0.40	0.3504	87	
RBLK c	NV/0-12"	-	<0.02	-	
EI9106-C1	NV/12-24"	-	<0.02	-	(<0.02)
EI9106-C2	NV/12-24"	-	<0.02	-	(<0.02)
EI9106-S3	NV/12-24"	0.04	0.0358	90	(0.0019) d
EI9106-S4	NV/12-24"	0.04	0.0324	81	(0.0018) d
EI9108-C	NV/24-36"	-	<0.02	-	<0.02
EI9108-C2	NV/24-36"	-	<0.02	-	<0.02
EI9108-S3	NV/24-36"	0.04	0.0383	96	
EI9108-S4	NV/24-36"	0.04	0.0367	92	
			Mean =	84.8%	
			Standard Deviation =	± 6.7	
			(n=10)		

a The control contribution of 0.0046 ppm is subtracted from the % recovery value.
b Realiquot; The original sample yielded low recovery (%) due to in-lab sample loss.
c Reagent blank.
d Estimated value.
( ) Parenthetical information represents complementary fraction.

Table IV

Summary of Method Validation

North Carolina Soil

Analyte	Soil Depth	Reps	Avg. % Recovery
Pyridate	0-12"	6	88.3
Pyridate	12-24"	2	79.3
Pyridate	24-36"	2	80.5
O-Methyl	0-12"	6	85.3
O-Methyl	12-24"	2	90.5
O-Methyl	24-36"	2	81.5
CL-9673	0-12"	6	81.5
CL-9673	12-24"	2	85.5
CL-9673	24-36"	2	94.3

Table V

RHEODYNE VALVE SWITCHING SCHEDULE

PROGRAM EVENTS TABLE*

TIME	EVENT	ACTION
INIT	S1	OFF
INIT	S2	ON
INIT	S3	OFF
INIT	S4	ON
1.50	S3	ON
1.50	S4	OFF
3.70	S1	ON
3.70	S2	OFF
9.00	S1	OFF
9.00	S2	ON
40.00	S1	OFF
40.00	S2	OFF
40.00	S3	OFF
40.00	S4	OFF

NOTES:

Time 1.80 - 3.70 is the heart-cut time.

Time 9.00 - 15.00 is for column re-equil.

* Representative values, times may vary.

CHOICE OF EVENTS:

S1-4 = Switches 1-4
S5 = Alarm
S6 = Sparge ml/min

CHOICE OF ACTIONS:

0 = OFF 1 = ON 2 = PULSE

Table VI

VALCO VALVE SWITCHING SCHEDULE

PROGRAM EVENTS TABLE*

TIME	EVENT	ACTION
[illegible]	S1	ON
[illegible]	S2	OFF
2.25	S2	ON
[illegible]	S1	OFF
11.00	S1	ON

NOTES:

Time 2.25 - 3.88 is the heart-cut time.

Time 11.00 - end of run is for column re-equilibration.

* Representative values, times may vary.

CHOICE OF EVENTS:

S1-4 = Switches 1-4
S5 = Alarm
S6 = Sparge ml/min

CHOICE OF ACTIONS:

0 = OFF 1 = ON 2 = PULSE

FIGURE 1

FLOW DIAGRAM FOR THE EXTRACTION
OF PYRIDA.E AND METABOLITES FROM SOIL

Parent/CM6 CL-9673

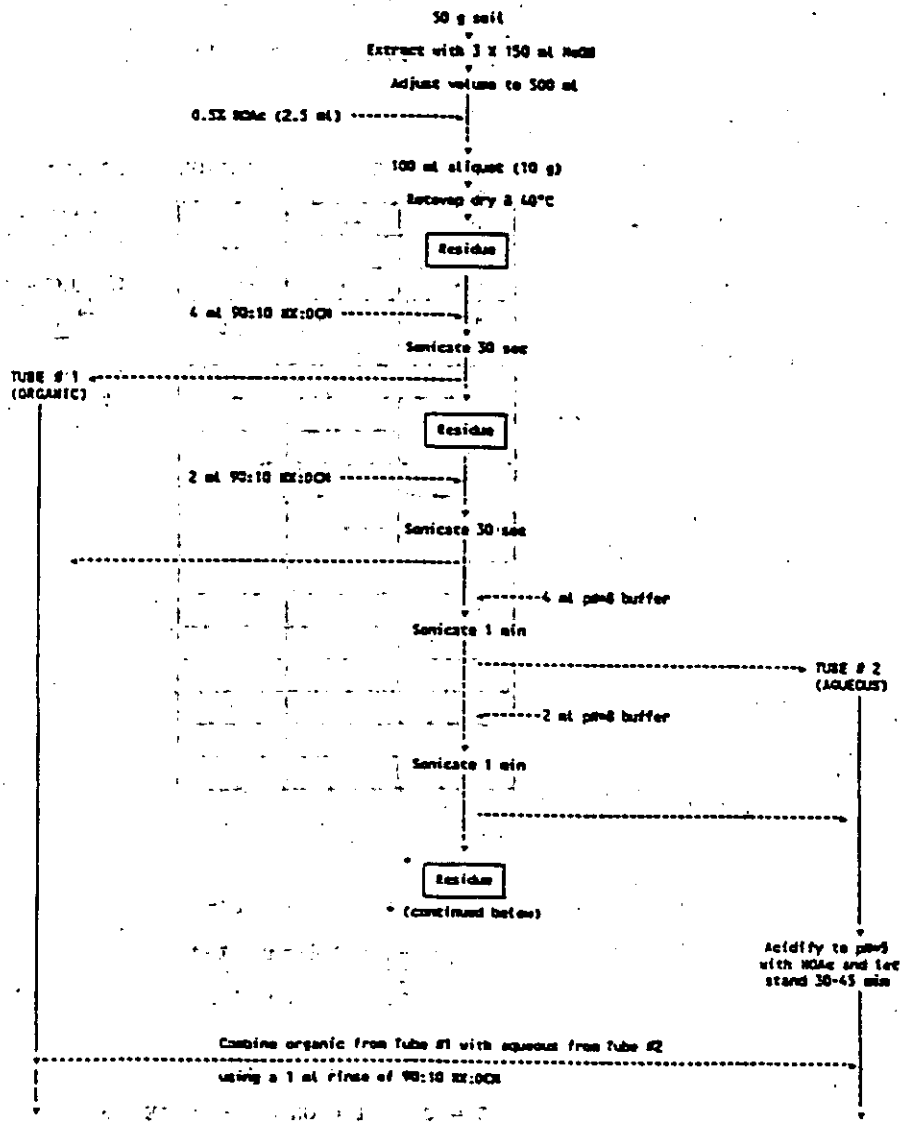
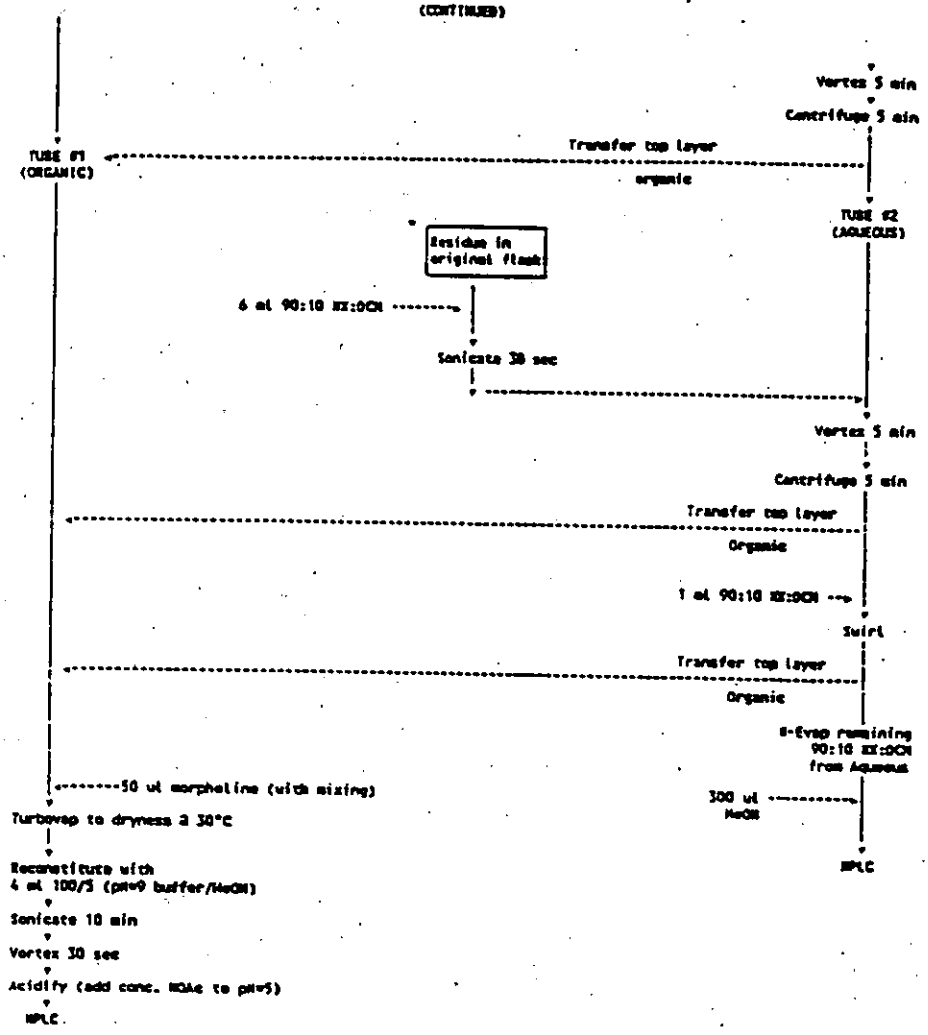


FIGURE 1

FLOW DIAGRAM FOR THE EXTRACTION
OF PYRIDATE AND METABOLITES FROM SOIL
(CONTINUED)



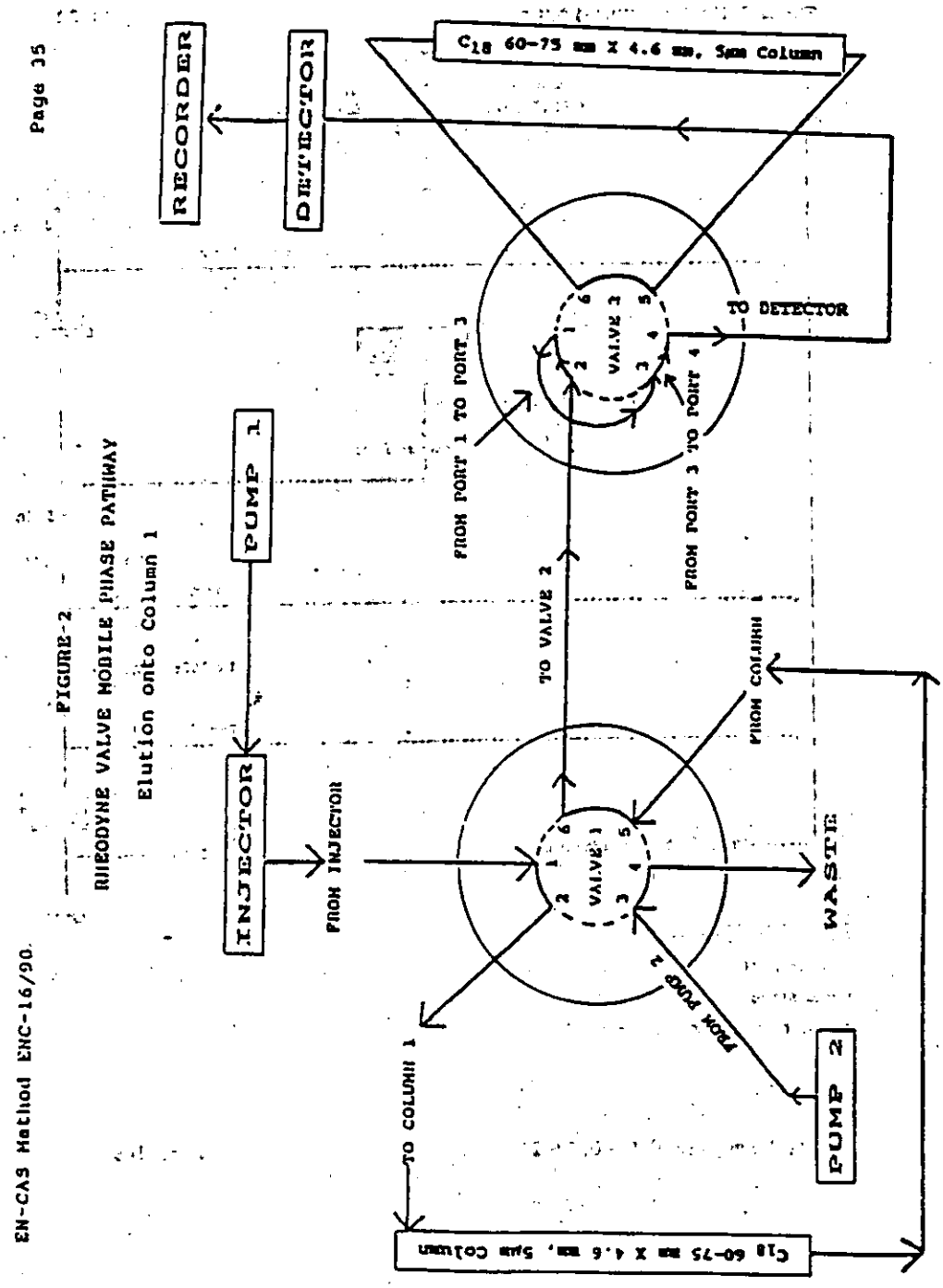


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FIGURE-2

RHEODYNE VALVE MOBILE PHASE PATHWAY

Elution onto Column 1

EN-CAS Method ENC-16/90.

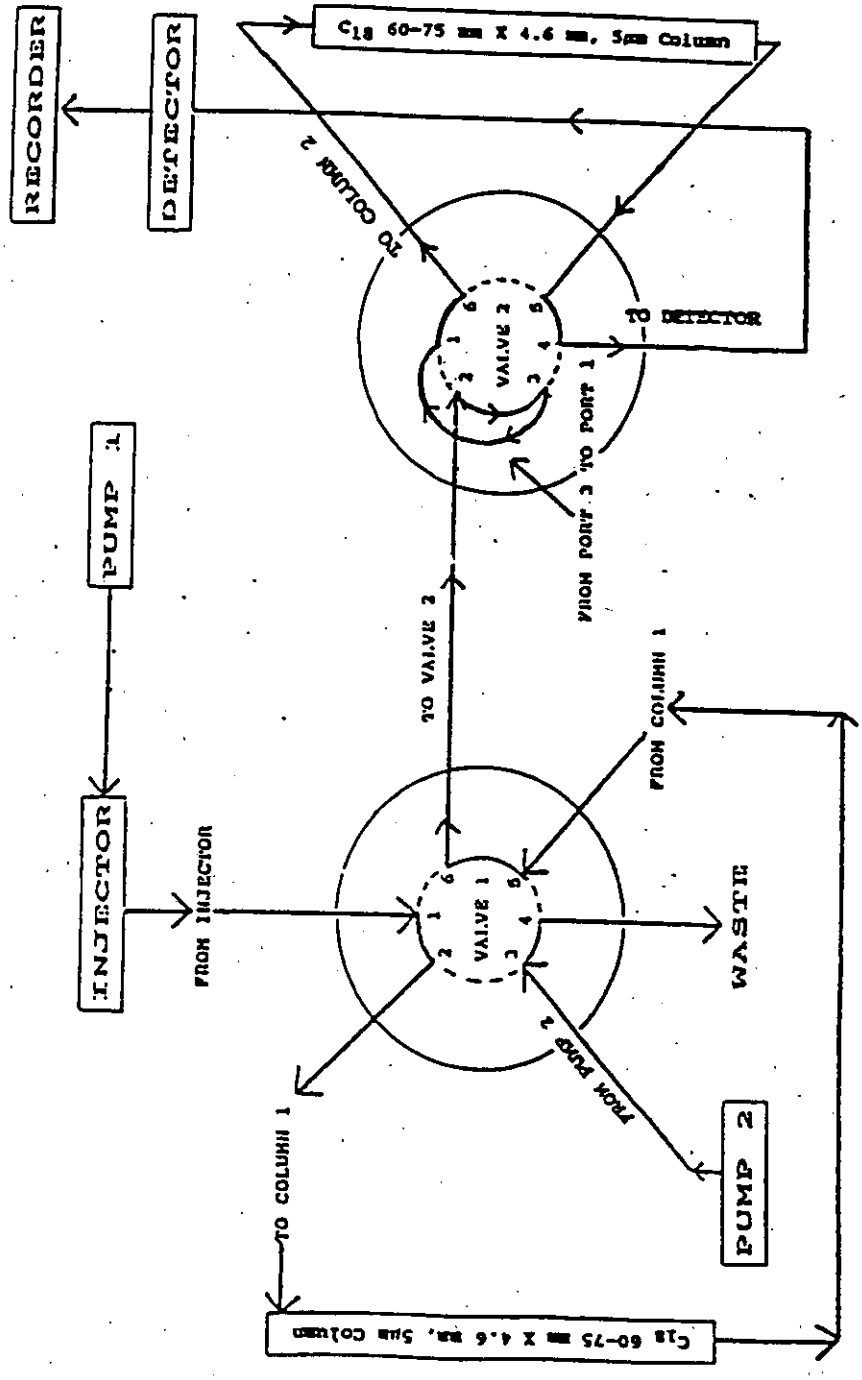
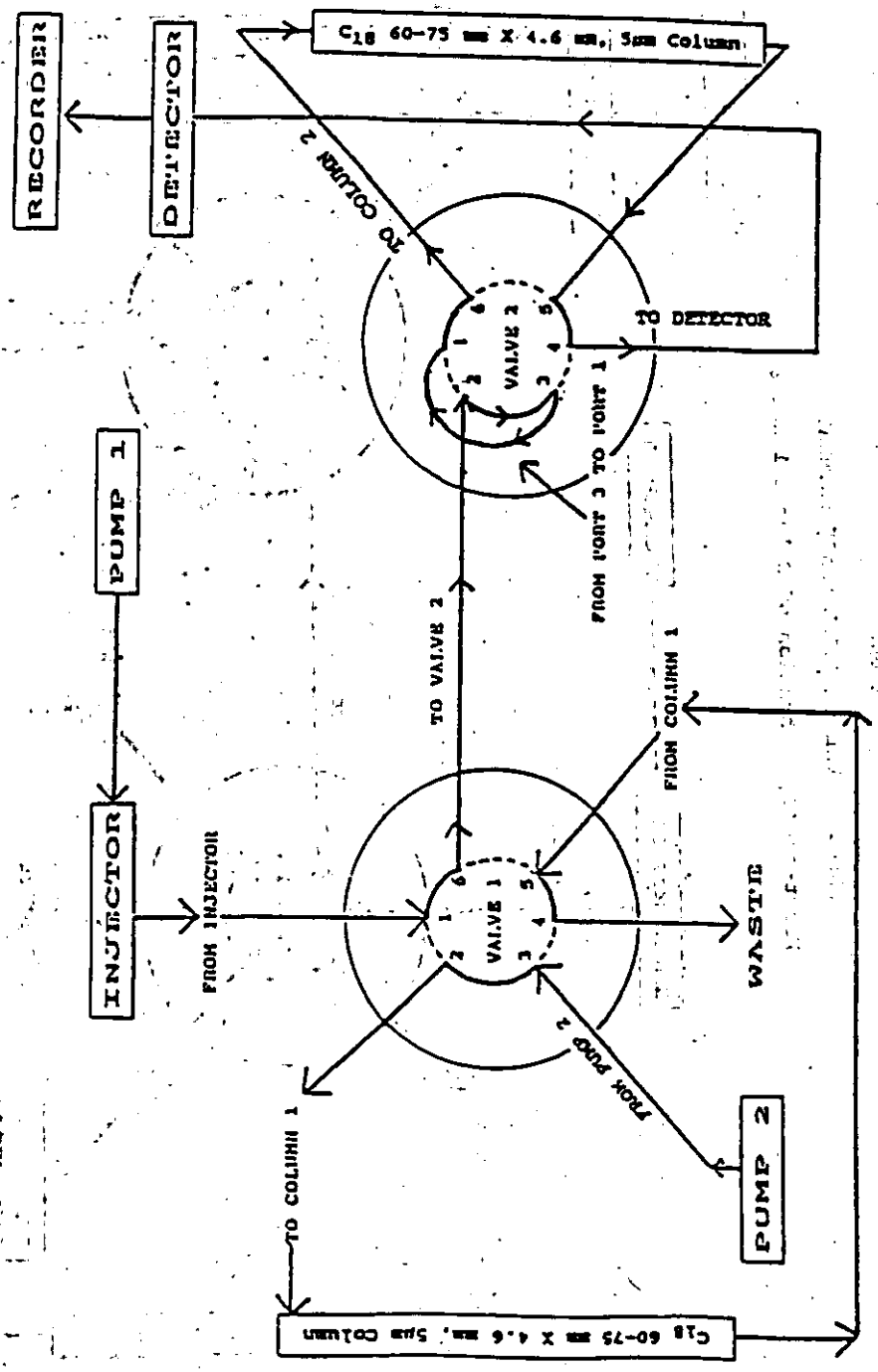


FIGURE 3

RHEODYNE VALVE MOBILE PHASE PATHWAY

Heart-cut/Equilibration of Columns 1 and 2

EN-CAS Method ENC-16/90



PIGURE 4

RHEODYNE VALVE MOBILE PHASE PATHWAY

Separation on Column 2/ Flushing Column 1

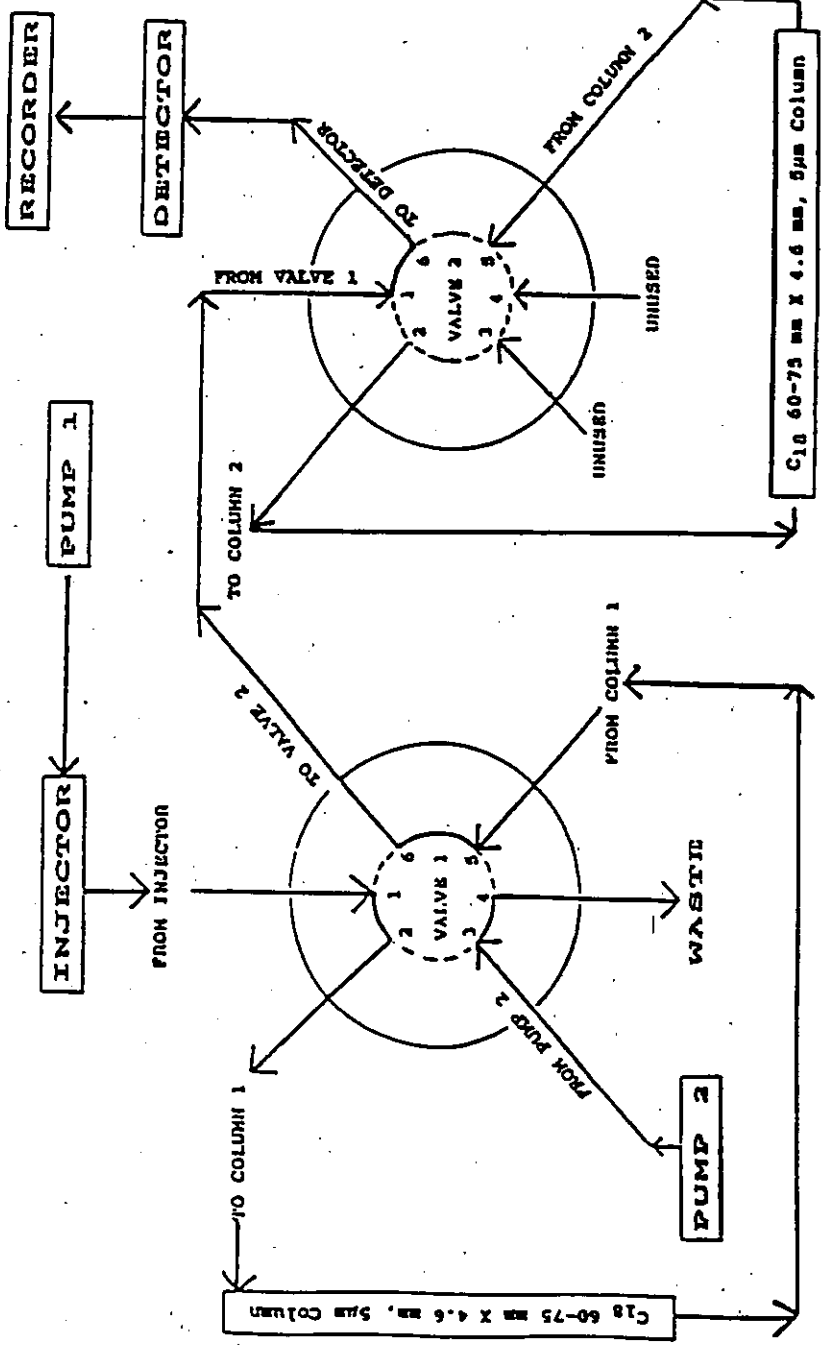


FIGURE 5

VALCO VALVE MOBILE PHASE PATHWAY

Elution onto Column 1

EN-CAS Method ENC-16/90

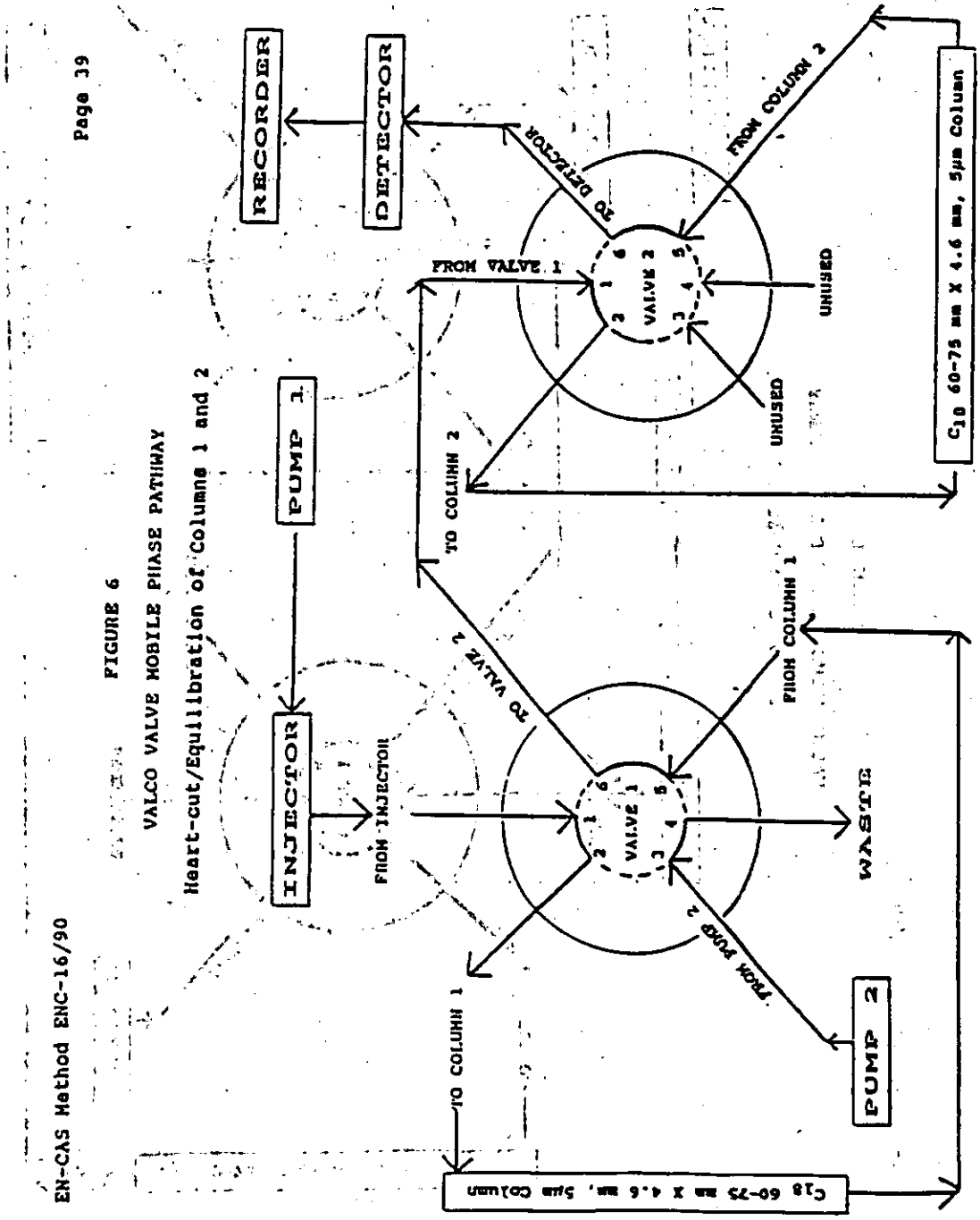


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FIGURE 6

VALCO VALVE MOBILE PHASE PATHWAY

Heart-cut/Equilibration of Columns 1 and 2

EN-CAS Method ENC-16/90

FIGURE 7

VALCO VALVE MOBILE PHASE PATHWAY

Separation on Column 2/ Flushing Column 1

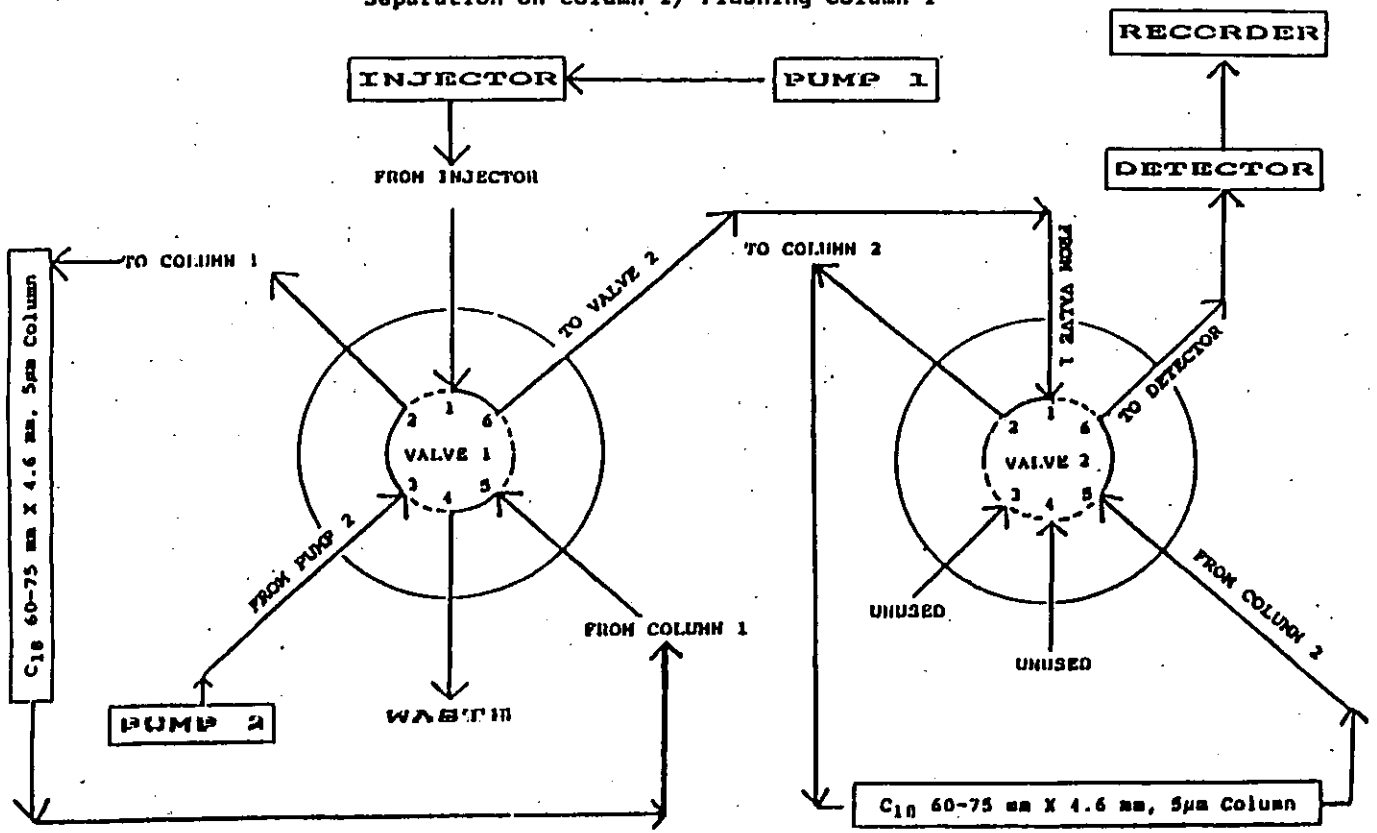
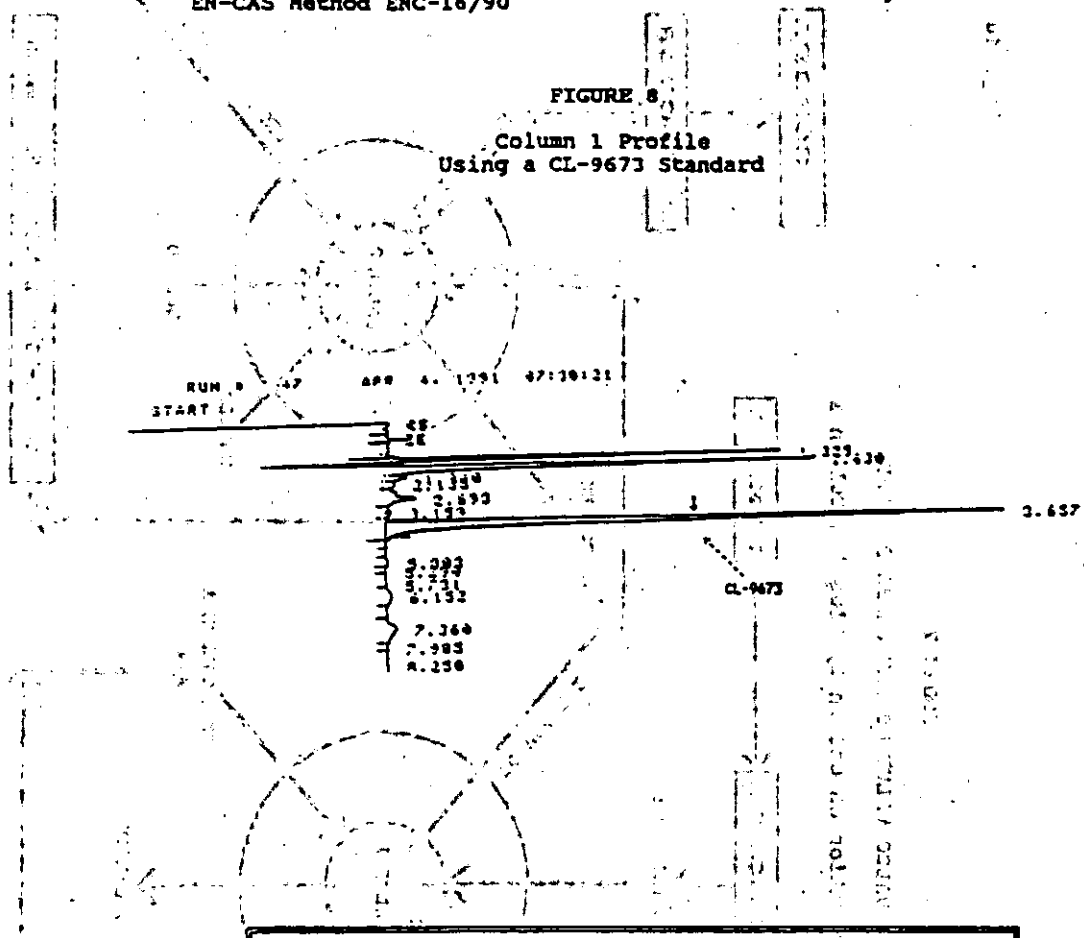


FIGURE 8

Column 1 Profile
Using a CL-9673 Standard



Peak width @ 1/2 pk. ht. = 0.9 mm

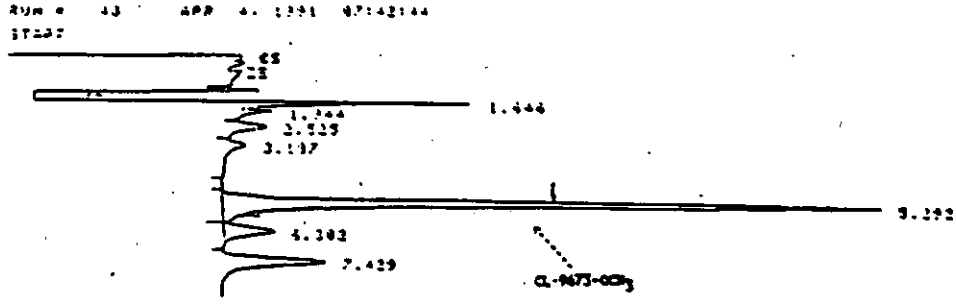
0.9 mm x 1.5 = 1.4 mm or 0.14 cm

Divide by chart speed: $\frac{0.14 \text{ cm}}{0.5 \text{ cm/min.}}$ = 0.28 min.

End the heart-cut: 3.657 min. + 0.28 min. = 3.937 min.

FIGURE 9

Column 1 Profile
Using An CL-9673-O-methyl Standard



Peak width @ 1/2 pk. ht. = 1 mm

1 mm x 1.5 = 1.5 mm or 0.15 cm

Divide by chart speed: $\frac{0.15 \text{ cm}}{0.5 \text{ cm/min.}} = 0.3 \text{ min}$

End the heart-cut: 5.292 min. + 0.3 min. = 5.592 min

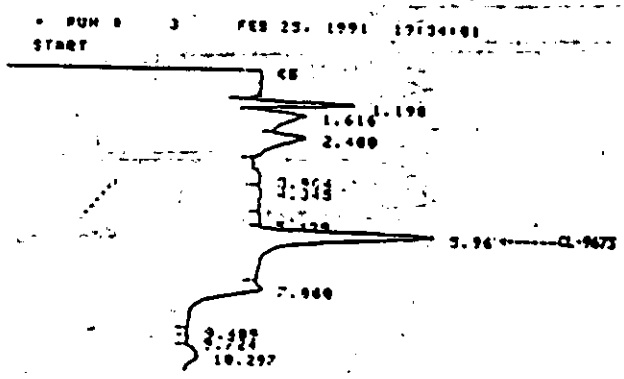
FIGURE 10

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.10 ug/ml CL-9673



25 ng injected

LC Run # 31718, dated 2/25/91

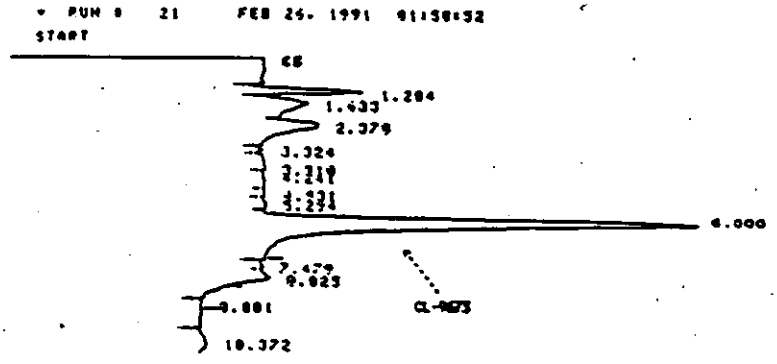
FIGURE 11

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.25 ug/ml CL-9673



62.5 ng injected

LC Run # 31718, dated 2/26/91

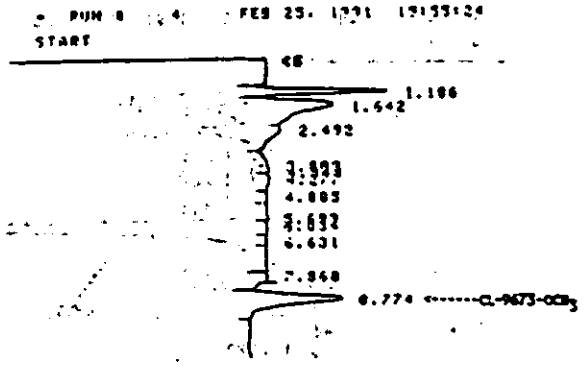
FIGURE 12

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.10 ug/ml CL-9673-OCH_3



25 ng injected

LC Run # 31718, dated 2/25/91

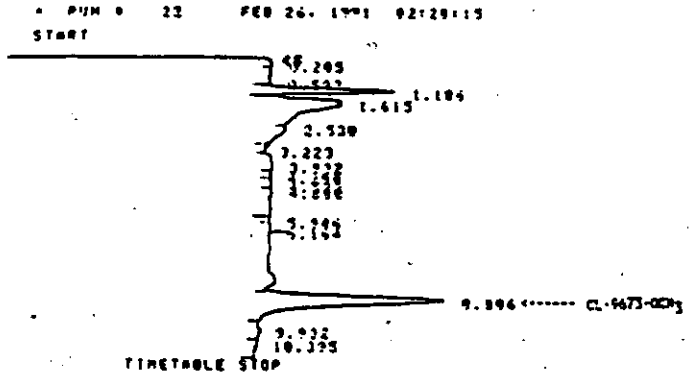
FIGURE 13

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.25 ug/ml CL-9673-OCH_3



62.5 ng injected

LC Run # 31718, dated 2/26/91

FIGURE 14

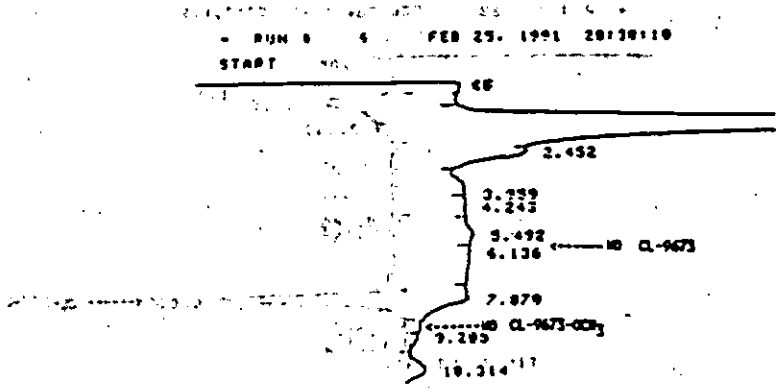
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control Sample

Organic Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-C2

<0.02 ppm Pyridate as CL-9673 Found
<0.02 ppm CL-9673-OCH_3 Found

4 ml final volume, 250 ul injected
LC Run # 31718, dated 2/25/91

FIGURE 15

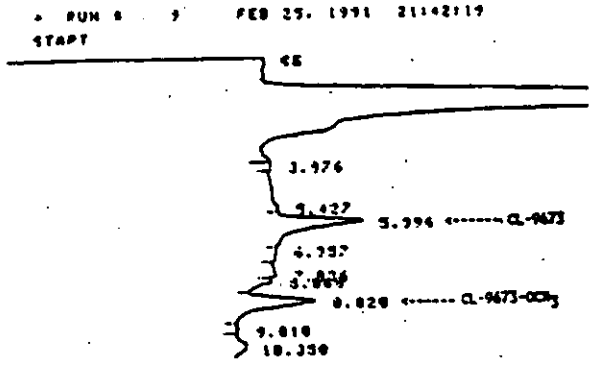
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.04 ppm Pyridate/CL-9673-OCH_3

Organic Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-S1

0.0366 ppm Pyridate as CL-9673 Found, 92% Recovery
0.0342 ppm CL-9673-OCH_3 Found, 85% Recovery

4 ml final volume, 250 ul injected
LC Run # 31718, dated 2/25/91

FIGURE 16

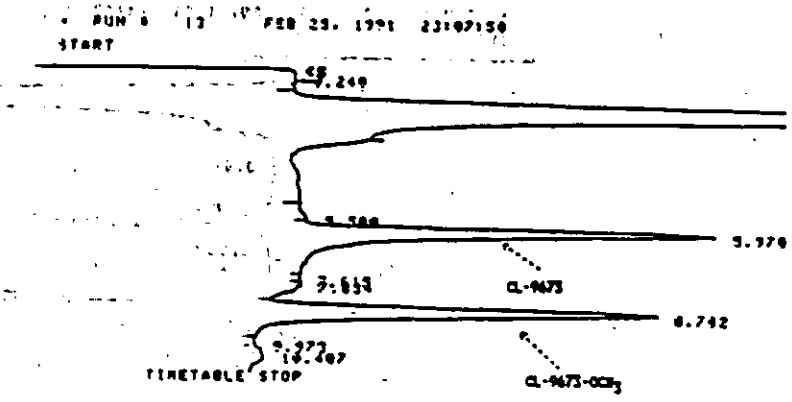
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.20 ppm Pyridate/CL-9673-OCH_3

Organic Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-S3

0.1751 ppm Pyridate as CL-9673 Found, 88% Recovery
0.1815 ppm CL-9673-OCH_3 Found, 91% Recovery

4 ml final volume, 250 ul injected
LC Run # 31718, dated 2/25/91

FIGURE 17

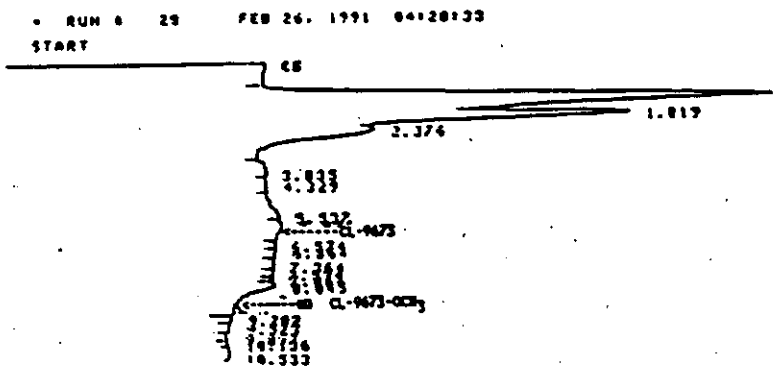
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control Sample

Aqueous Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-C2 (AQ)

<0.02 ppm CL-9673 Found

4 ml final volume, 250 ul injected
LC Run # 31721, dated 2/26/91

FIGURE 18

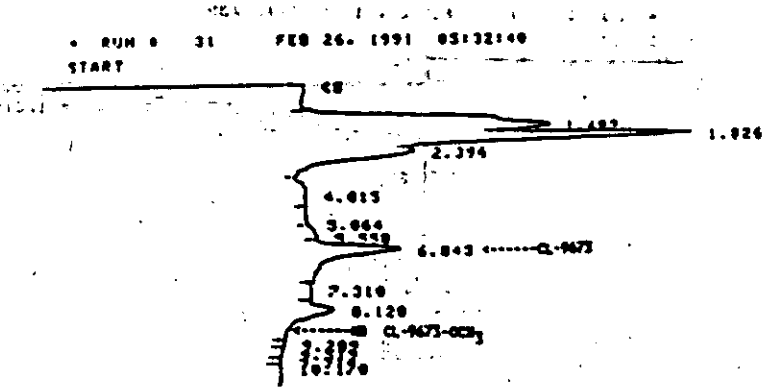
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.04 ppm CL-9673

Aqueous Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-S8 (AQ)

0.0326 ppm CL-9673 Found, 73% Recovery*

4 ml final volume, 250 ul injected
LC Run # 31721, dated 2/26/91

*The % recovery is corrected for the control contribution of 0.0046 ppm.

FIGURE 19

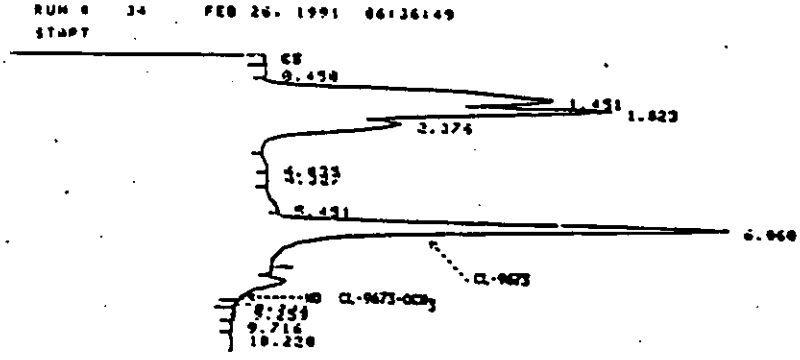
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.20 ppm CL-9673

Aqueous Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-S10 (AQ)

0.1670 ppm CL-9673 Found, 82% Recovery

4 ml final volume, 250 ul injected
LC Run # 31721, dated 2/26/91

FIGURE 20

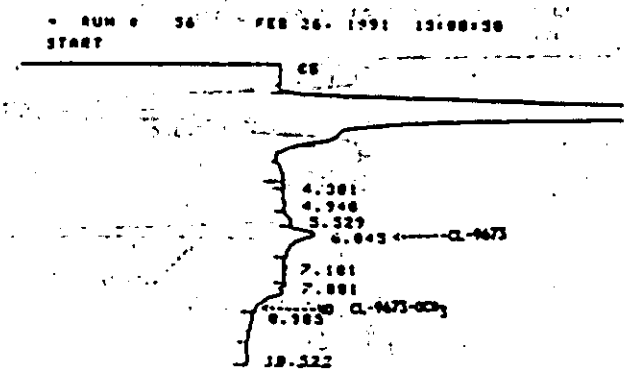
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.20 ppm CL-9673

Organic Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-S10 (Organic)

(Note: Complimentary fraction of sample fortified with free CL-9673 only, showing 3% remaining in the organic phase.).

4 ml final volume, 250 ul injected
LC Run # 31718, dated 2/26/91

FIGURE 21

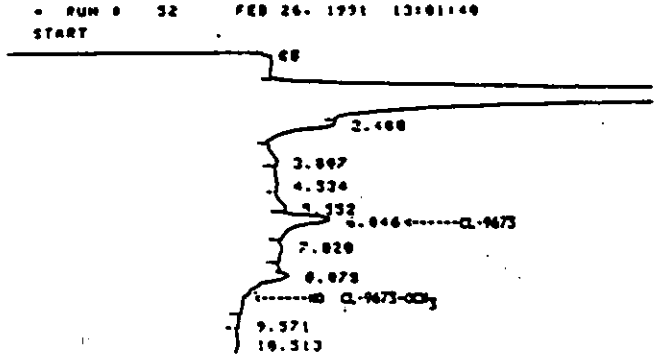
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.40 ppm CL-9673

Organic Fraction

0-12" Depth



EN-CAS Sample ID#: EI9104-S11 (Organic)

(Note: Complimentary fraction of sample fortified with free CL-9673 only, showing 5% remaining in the organic phase.)

4 ml final volume, 250 ul injected
LC Run # 31718, dated 2/26/91

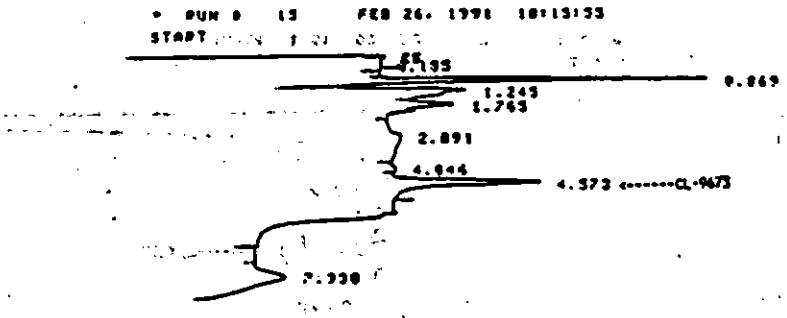
FIGURE 22

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.05 ug/ml CL-9673



12.5 ng injected

LC Run # 31719, dated 2/26/91

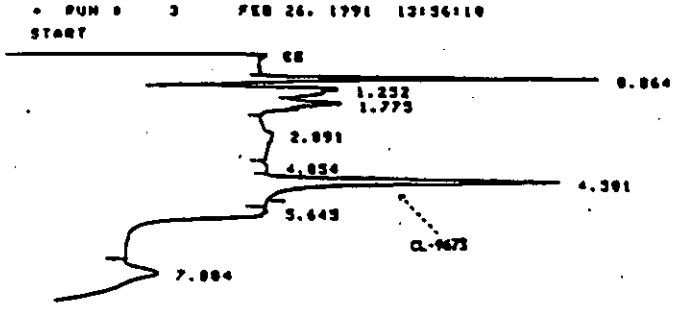
FIGURE 23

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.10 ug/ml CL-9673



25 ng injected

LC Run # 31719, dated 2/26/91

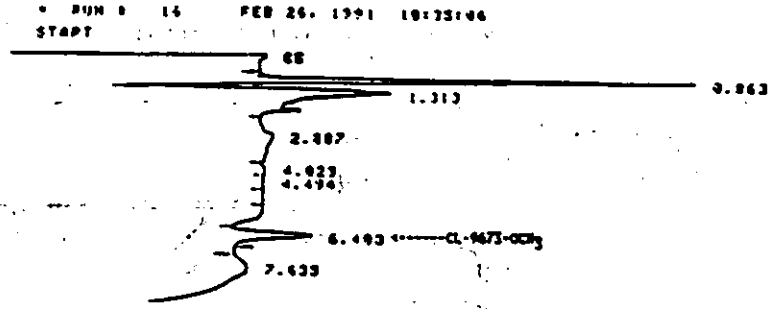
FIGURE 24

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.05 ug/ml CL-9673-OCH_3



12.5 ng injected

LC Run # 31719, dated 2/26/91

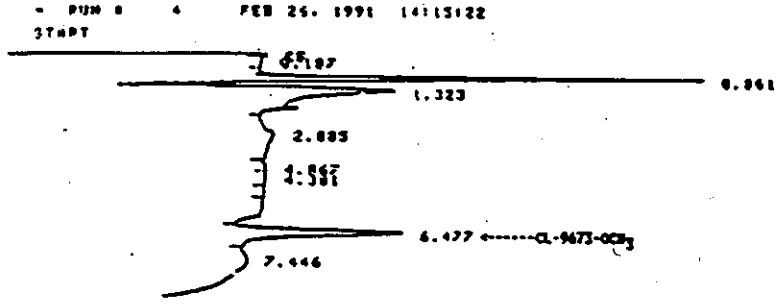
FIGURE 25

Typical Chromatogram

Pyridate Soil Analysis

L.C. Standard

0.10 ug/ml CL-9673-OCH_3



25 ng injected

LC Run # 31719, dated 2/26/91

FIGURE 26

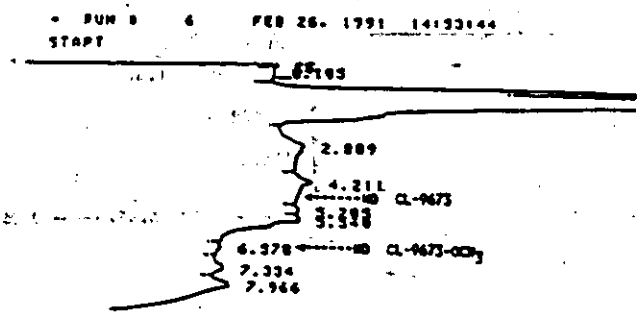
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control Sample

Organic Fraction

12-24" Depth



EN-CAS Sample ID#: EI9106-C2

<0.02 ppm Pyridate as CL-9673 Found
<0.02 ppm CL-9673-OCH_3 Found

4 ml final volume, 250 ul injected
LC Run # 31719, dated 2/26/91

FIGURE 27

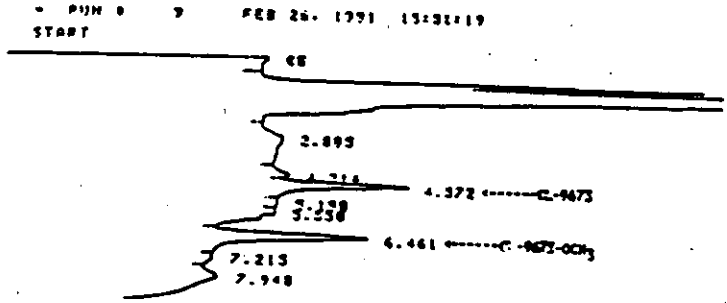
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.04 ppm Pyridate/CL-9673-OCH_3

Organic Fraction

12-24" Depth



EN-CAS Sample ID#: EI9106-S1

0.0328 ppm Pyridate as CL-9673 Found, 82% Recovery
0.0367 ppm CL-9673-OCH_3 Found, 92% Recovery

4 ml final volume, 250 ul injected
LC Run # 31719, dated 2/26/91

FIGURE 28

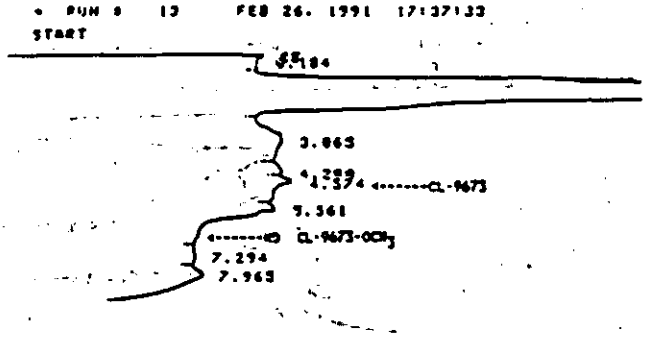
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.04 ppm CL-9673

Organic Fraction

12-24" Depth



EN-CAS Sample ID#: EI9106-S3

(Note: Complementary fraction of sample fortified with free CL-9673 only, showing 5% remaining in the organic phase.)

4 ml final volume, 250 ul injected
LC Run # 31719, dated 2/26/91

FIGURE 29

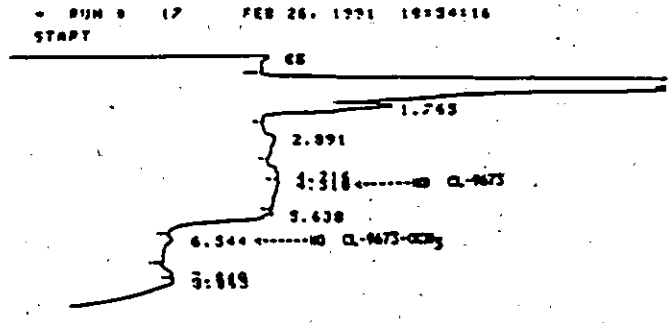
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control Sample

Aqueous Fraction

12-24" Depth



EN-CAS Sample ID#: EI9106-C1 (AQ)

<0.02 ppm CL-9673 Found

4 ml final volume, 250 ul injected
LC Run # 31719, dated 2/26/91

FIGURE 30

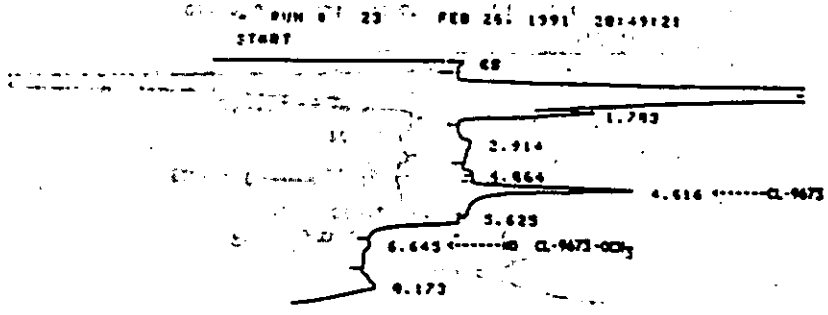
Typical Chromatogram

North Carolina Pyridate Soil Analysis

Control + 0.04 ppm CL-9673

Aqueous Fraction

12-24" Depth



EN-CAS Sample ID#: EI9106-S3 (AQ)

0.0358 ppm CL-9673 Found, 90% Recovery

4 ml final volume, 250 ul injected
LC Run # 31719, dated 2/26/91

APPENDIX C

EN-CAS METHOD NO. ENC-16/90
METHOD ADDENDUM 1

EN-CAS METHOD NO. ENC-16/90
METHOD ADDENDUM 1

Analytical Procedure for the Determination of Pyridate and Its Primary Metabolites CL-9673 and CL-9673-O-methyl in Soil

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Total Pages = 3

EN-CAS METHOD NO. ENC-16/90	AUTHOR(S) Wayne Barker	DATE ISSUED: 10/8/91 REVISIONS:
TITLE: Analytical Procedure for the Determination of Pyridate and Its Primary Metabolites CL-9673 and CL-9673-O-methyl in Soil	QA APPROVAL 10/8/91 MGMT APPROVAL 10/08/91	

1.0 INTRODUCTION

1.1 Scope

This method addendum is written in order to describe modifications to method ENC-16/90 for soils from sites other than those in North Carolina. The five additional sites are located in Georgia, California, Wisconsin, Iowa and Illinois.

1.2 Principle

The method as described in EN-CAS Method No. ENC-16/90 is unchanged with the exception of a modification that is made for the Georgia, California, Iowa, Illinois and Wisconsin sites. For these sites, the pH of the aqueous fraction of the samples is adjusted to 4.0-4.5 with acetic acid (vs pH 5.0 in the North Carolina site, according to section 8.3 in the method) prior to the partition step.

A further modification is made for the Georgia site only. The pH of the aqueous fraction of the Georgia samples is adjusted to 4.0 with acetic acid prior to injection on the HPLC (section 8.5 in the method).

These changes are made to improve the separation of Pyridate from Cl-9673 at the partition step, and also to counteract an unknown soil matrix component that caused a reduced Cl-9673 recovery in some soils.

2.0 REFERENCES

EN-CAS Method No. ENC-16/90, Analytical Procedure for the Determination of Pyridate and its Primary Metabolites CL-9673 and CL-96710-O-methyl in Soil, issued 4/25/91.