Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name:	Siduron
MRID # :	433279-03
Matrix:	Water
Analysis:	HPLC/UV

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

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

433279-03

STUDY TITLE

Siduron Technical:
Analytical Method Validation
in Freshwater

AUTHOR

Kelly L. Eyler

STUDY REPORT DATE

May 10, 1994

SPONSOR

Gowan Company
1644 Engler Avenue
Yuma, Arizona 85366-5569

PERFORMING LABORATORY

Toxikon Environmental Science
106 Coastal Way
Jupiter, Florida 33477

LABORATORY PROJECT ID

J9403003b

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Test Substance: Siduron Technical

Title: Siduron Technical: Analytical Method Validation in Freshwater

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

Sponsor: Gowan Company

Sponsor Representative: Robert E. Hawk Date: May 16, 1994
Signature
Robert E. Hawk

STATEMENT OF GLP COMPLIANCE

Test Substance: Siduron Technical

Title: Siduron Technical: Analytical Method Validation in Freshwater

This study was conducted in accordance with published Good Laboratory Practices (GLP) regulations for tests of substances regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) by the U.S. Environmental Protection Agency (40 CFR Part 160).

Kelly L. Eyler — 5-10-94
Kelly L. Eyler, Study Director — Date

Robert E. Hawk — May 16, 1994
Sponsor GOWAN COMPANY — Date

Robert E. Hawk — May 16, 1994
Submitter GOWAN COMPANY — Date

STATEMENT OF QUALITY ASSURANCE

Test Substance: Siduron Technical

Title: Siduron Technical: Analytical Method Validation in Freshwater

Test data were reviewed by the Quality Assurance Unit to assure that standard operating procedures and the protocol developed for this study were followed. This report is an accurate reflection of the raw data.

Type of Audit	Date of Audit	Date Reported to Study Director and Management
In-Study Audit:	04-11-94	04-12-94
Study Data Review:	05-06-94	05-06-94
Draft Report Review:	05-06-94	05-06-94
Final Report Review:	05-10-94	05-10-94

Tangela Y. Baker
Quality Assurance Auditor
Toxikon Environmental Sciences

Date 5/10/94

LIST OF SCIENTIFIC PERSONNEL

Test Substance: Siduron Technical

Title: Siduron Technical: Analytical Method Validation in Freshwater

Chemist: Kelly Eyler

Study Director: Kelly Eyler

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1.0 INTRODUCTION

An analytical method validation study was conducted at Toxikon Environmental Sciences (TES), Jupiter, Florida, to determine the precision and accuracy of a procedure to analyze Siduron Technical in freshwater. Du Pont methods HLR 39-89 and HLR 821-88, received from Gowan Company, were modified at TES with the objective of minimizing sample preparation steps and using standard laboratory materials.

Quantitation of Siduron Technical was performed by liquid chromatography (LC) using a UV/VIS detector and the external standard technique. The method was validated by fortifying laboratory freshwater with Siduron Technical at two concentrations which encompass the range of test concentrations expected to be utilized in toxicity tests of freshwater organisms. This study was conducted April 11, 1994.

All data related to this study will be archived at Gowan Company, Yuma, Arizona.

2.0 MATERIALS AND METHODS

2.1 Test Methods

The methods for the analytical validation of Siduron Technical in freshwater were those described in Toxikon Environmental Sciences' test protocol entitled: "Siduron Technical: Analytical Method Validation In Freshwater."

2.2 Apparatus And Materials

High Pressure Liquid Chromatograph: Shimadzu LC600

HPLC Detector: Shimadzu SPD10A (235 nm)

Autosampler: Perkin Elmer ISS 200 (20-µL injection volume)

HPLC Column: Zorbax C18, 4.6 mm x 25 cm (#F 38085)

Volumetric Flasks: 10-, 50-, and 100-milliliter (mL), class A with ground glass stoppers

Volumetric Pipettes: 1-, 2-, and 5-mL capacity, calibrated to deliver, and 500-µL glass syringes

Glassware: General assortment of laboratory glassware

Syringes: 5-cc plastic disposable

Syringe Filters: Gelman Acrodisc 13 mm, 0.45 µm PTFE membrane

Solvents and Reagents:

a. Water: Modulab PureOne (TES)
b. Acetonitrile HPLC grade (B&J)

Liquid Chromatographic Mobile Phase (HPLC): Added 700 mL acetonitrile and 300 mL water to a 1-L flask. Degassed by magnetic stirring under a vacuum.

Analytical Standard Compound: Siduron, Lot No. E62741-145, 99.7% (Appendix A)

Technical Standard Compound: Siduron, Lot Nos. 051490-94 , 051590-95, 051590-96 & 051690-97, mean purity of 97.75% (Appendix A) (Both compounds were received from Du Pont)

Matrix: Laboratory freshwater with the following characteristics: pH 7.5; 23°C; hardness 72 mg/L

2.3 Preparation Of Standard Solutions

A primary analytical stock solution was prepared by weighing 0.0103 gram (g) of Siduron Analytical (99.7% purity) into a 100-mL volumetric flask and bringing to volume with acetonitrile. The solution was thoroughly mixed. The resulting concentration of this primary analytical stock solution was 102.7 mg/L Siduron. A secondary analytical stock was prepared by pipetting 5.0 mL of the primary analytical stock into a 50-mL volumetric flask and bringing to volume with mobile phase. The solution was thoroughly mixed. The resulting concentration of this secondary analytical stock was 10.27 mg/L. A series of five working calibration standards were prepared as shown in Table 1 by adding the appropriate volumes of the secondary analytical stock solution to 10-mL volumetric flasks and bringing to volume with mobile phase.

A primary technical stock solution was prepared by weighing 0.0326 g of Siduron Technical (97.75% purity) into a 100-mL volumetric flask and bringing to volume with acetonitrile. The solution was thoroughly mixed. The resulting concentration of this primary technical stock solution was 318.7 mg/L Siduron. A secondary technical stock solution was prepared by adding 0.75 mL of the primary technical stock to a 10-mL volumetric flask and bringing to volume with acetonitrile. The solution was thoroughly mixed. The resulting concentration of this secondary technical stock solution was 23.90 mg/L Siduron.

2.4 Preparation Of Spike Samples

Spike samples were prepared by adding 1.0 mL of the 23.90 mg/L secondary technical stock solution to a 50.0-mL volumetric flask and bringing to volume with freshwater. The resulting concentration of the low level spike samples was 0.478 mg/L.

Spike samples were also prepared by adding 1.0 mL of the 318.7 mg/L primary technical stock solution to a 10.0-mL volumetric flask and bringing to volume with freshwater. The resulting concentration of the high level spike samples was 31.87 mg/L.

Each spike level was prepared in triplicate. A matrix blank was prepared from an unfortified 10-mL aliquot of freshwater. All spike samples and the matrix blank were filtered through a 0.45-µm PTFE syringe filter before dilution into the calibration range (if necessary) and LC analysis.

2.5 Liquid Chromatographic Analysis

The LC600 pump and SPD10 A UV/VIS detector were set with the following conditions:

Column:	Zorbax C18 4.6-mm x 25-cm column (room temperature)
Detector Wavelength:	235 nm
Mobile Phase:	70:30 ACN:H_2O
Flow Rate:	1.0 mL/min, isocratic
Chart Speed:	0.5 cm/min

After equilibration of the system and attainment of a stable baseline on the integrator, quality control samples (method blank and calibration standards) were analyzed along with the validation spike samples to assess the accuracy and precision of the method. A matrix blank of unspiked freshwater was analyzed to determine the limit of quantitation.

2.6 Quantitation

The standard response curve (linear regression curve) of Siduron concentration versus peak area (integrator response) was generated from the data obtained during the validation (Figure 1). The equation of the curve is:

Siduron mg/L = (Peak Area + 115.19)/43952,

with a correlation coefficient of 1.000. The Siduron concentration found in the samples was calculated using the following equation:

mg/L Siduron from std curve * dil factor = mg/L Siduron

2.7 Example Calculation

Run Date: April 11, 1994

Run Report#: 13 (MVB5; 31.87 mg/L)

Response = 136932

Dilution Factor = 10X

Siduron mg/L = (136932 + 115.19)/43952

= 3.118 mg/L * 10 = 31.18 mg/L

2.8 Limit of Detection

The limit of detection for Siduron was calculated from a matrix blank and a low concentration standard (0.308 mg/L Siduron). The signal(S)-to-noise(N) ratio for the 0.308 mg/L standard and matrix blank was 36. Extrapolation to a S/N ratio of 3 is 0.026 mg/L which is the limit of detection (LOD).

Matrix blank = 3.0 mm S/N =36
0.308 mg/L = 108.0 mm

3/36 = LOD mg/L / 0.308 mg/L LOD = 0.026 mg/L

3.0 RESULTS AND DISCUSSION

Recovery data from the fortified freshwater samples analyzed during this method validation study are presented in Table 2. Samples with a spike concentration of 0.478 mg/L had an average recovery of 93% with a standard deviation of 0.6%. Samples with a spike concentration of 31.87 mg/L had an average recovery of 99% with a standard deviation of 1.2%. The overall average recovery was 96% with a standard deviation of 3.4%. The limit of detection was 0.026 mg/L.

4.0 CONCLUSION

The method described is suitable for the analysis of Siduron in freshwater over a concentration range of 0.478 to 31.87 mg/L.

Table 1. Preparation of Working Calibration Standards for Siduron

Standard Designation	Volume of Standard (mL)	Stock Concentration (mg/L)	Final Volume (mL)	Standard Concentration (mg/L)
Std 1	0.30	10.27	10	0.308
Std 2	1.00	10.27	10	1.027
Std 3	2.00	10.27	10	2.054
Std 4	3.00	10.27	10	3.081
Std 5	4.00	10.27	10	4.108

Table 2. Recovery Data for Siduron From Freshwater During Method Validation

Spike Level	Dilution Factor	mg/L Added	mg/L Found	Percent Recovery*
Method Blank	1X	0.0	<0.026	N/A
Spike Rep 1	1X	0.478	0.445	93
Spike Rep 2	1X	0.478	0.445	93
Spike Rep 3	1X	0.478	0.448	94
		Mean ± Standard Deviation = 93 ± 0.6%		
Spike Rep 1	10X	31.87	31.18	98
Spike Rep 2	10X	31.87	31.83	100
Spike Rep 3	10X	31.87	31.78	100
		Mean ± Standard Deviation = 99 ± 1.2%		
		Grand Mean ± Standard Deviation = 96 ±3.4%		

*Percent Recovery = (mg/L found ÷ mg/L added) X 100%

Figure 1. Calibration Curve for Validation of Siduron in Freshwater

Siduron Method Validation J9403003b
KE April 11, 1994

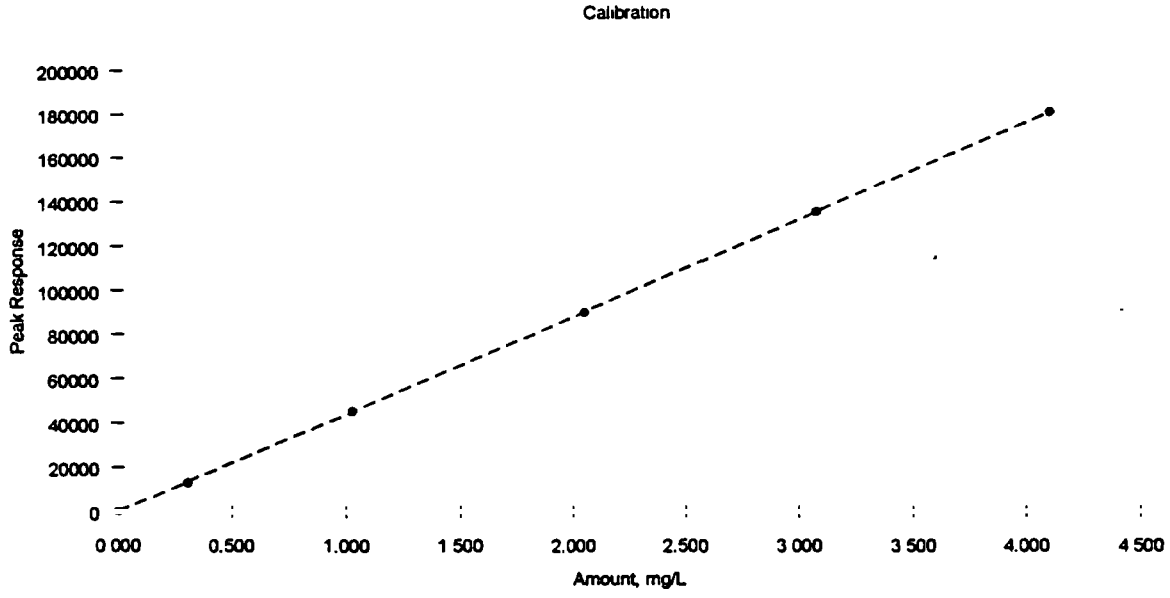
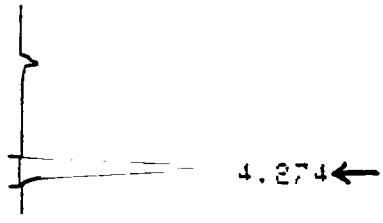


Figure 2. Typical Chromatogram of Siduron (arrow denotes the retention time of siduron)

a. Standard-2: 1.027 mg/L as Siduron



b. Mobile Phase Blank

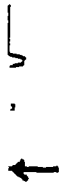
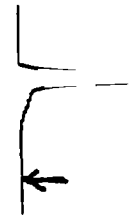


Figure 2 (Cont.) Typical Chromatogram of Siduron

c. MVB5: high spike replicate 1 Siduron; 31.87 mg/L as Siduron in freshwater; 10X dilution factor

d. MVB1: Freshwater method blank; no dilution



APPENDIX A

CERTIFICATES OF ANALYSIS

E. I. du Pont de Nemours & Company
Experimental Station
Wilmington Delaware

Certificate of Analysis

Shipping Request #: 4054

Date Issued: 24-Feb-1994

IN # Z1318- Dash # 70 Standard Type: PRIMARY

Percent Purity: 99.7 Ref. # E62741-145

Storage Advice: ROOM TEMPERATURE/ CABINET

This material should meet the assigned purity for a period of one year when it has been stored appropriately.

At the end of one year, please contact the Analytical Standards Laboratory for recharacterization information.

DUPONT

AGRICULTURAL PRODUCTS
Experimental Station
P.O. Box 80402
Wilmington, Delaware 19880-0402

cc: R. M. Vaught
M. Wolters

February 23, 1994

Dr. G. Scott Ward
Laboratory Manager
Toxikon Environmental Sciences
106 Coastal Way
Jupiter, FL 33477

Dear Dr. Ward:

This letter accompanies a shipment of ~1.1 kg of siduron technical. This siduron sample is a composite of about equal portions of four batches, each assayed at 97.1-98.1% purity (see attached assay results). As best I can determine, there is no more siduron technical available within DuPont, so I hope this quantity will meet your research needs.

A 1-g sample of analytical standard siduron is being shipped separately.

Regards,

Barbara C. Rhodes
Research Chemist

ANALYSIS OF SIDURON SAMPLES SHIPPED DECEMBER 1992

ANALYSIS#	B# *	#	%INSOL	ASSAY	VOL	AMINE
~~F8575~~	~~A 050390~~	~~91~~	~~.19~~	~~97.3~~	~~0.0~~	~~0.06~~
~~F8580~~	~~A 050390~~	~~92~~	~~.08~~	~~97.7~~	~~0.0~~	~~0.06~~
~~F8594~~	~~050390~~	~~93~~	~~.08~~	~~97.0~~	~~0.0~~	~~0.04~~
F8607	051490	94	.32	97.1	0.09	0.06
F8612	051590	95	.15	98.1	0.09	0.10
F8618	051590	96	.25	97.9	0.08	0.09
F8622	051690	97	N.D.	97.9	0.0	0.09

Mean purity 97.75%

* B# = Analysis Date