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