

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 F8426 Technical in filtered and unfiltered saltwater. The analysis of F8426 Technical in saltwater required direct injection of aqueous samples (after dilution into 50:50 acetonitrile:water if necessary to stay within calibration range).

Quantitation of F8426 Technical was performed by liquid chromatography (LC) using a UV/VIS detector and external standardization. The method was first validated by fortifying filtered saltwater with F8426 Technical at two concentrations which encompassed the range of test concentrations expected to be utilized in toxicity tests with saltwater organisms. This study was conducted on April 1, 1996. Since in this validation a degradation of sample was observed, a second validation was conducted at two concentrations on April 23, 1996. Only the second validation of the filtered saltwater is described in this report. In the second validation of the filtered saltwater, as well as the unfiltered saltwater validation and all other work with F8426 Technical, all samples and standards were kept at 4°C until analysis to prevent this degradation of test substance.

A third validation was conducted by fortifying unfiltered saltwater with F8426 Technical at two concentrations which encompassed the range of test concentrations expected to be utilized in a toxicity test on the Eastern Oyster. This study was conducted on April 9, 1996.

2.0 MATERIALS AND METHODS

2.1 TEST METHODS

The analytical method for F8426 Technical was developed and validated in filtered and unfiltered saltwater at Toxikon Environmental Sciences following the test protocol entitled: "F8426 Technical: Analytical Method Validation In Filtered and Unfiltered Saltwater" (Appendix A).

2.2 TEST SUBSTANCE

The test substance, F8426 Technical (Lot No. PL93-356) was provided by FMC Corporation and received at Toxikon Environmental Sciences on February 16, 1996, in a plastic bottle labeled "F8426 Technical Herbicide: Reference: PL93-356; Amount 1 kg; Date 2-5-96." The substance was received as a viscous orange oil and stored in the dark at ambient room temperature. F8426 Technical was reported by FMC Corporation to be 90.3% pure and stable for at least 30 days (FMC Study No. P96-0125).

2.3 APPARATUS AND MATERIALS

HPLC Pump: Shimadzu LC-10AD

HPLC Detector: Shimadzu SPD 10A UV/VIS

Autoinjector: Waters Model 710B (100 μ L injection volume)

Data Acquisition System: Hewlett-Packard Model 3396 Series II

HPLC Column: Intersil 5 (C-8), 4.6-mm x 150-mm
5- μ m particle size

Solvents and Reagents:

a. Water (H₂O): HPLC Grade (JT Baker)

b. Acetonitrile (ACN): HPLC grade (B&J or JT Baker)

Liquid Chromatographic (HPLC) Mobile Phase: 60:40 ACN:H₂O,
degassed by continuous helium sparging

Matrices: Natural, filtered saltwater with the following
characteristics: salinity of 20 ‰; temperature 24°C

Natural, unfiltered saltwater with the following characteristics: salinity of 28-36 ‰; temperature 24°C

2.4 PREPARATION OF STANDARD SOLUTIONS

A primary test substance stock solution (2.49 mg ai/mL) was prepared by weighing 0.2760 gram (g) of F8426 Technical into a 100-mL volumetric flask and bringing to volume with ACN. The solution was thoroughly mixed. Two secondary stock solutions (0.100, 1.00 mg/mL) were prepared by adding, respectively, 4.02 mL and 40.2 mL of the primary stock solution into 100 mL volumetric flasks and bringing to volume with ACN. A series of five working calibration standards was prepared as shown in Table 1 by adding the appropriate volumes of the secondary test substance stock solutions to 100-mL volumetric flasks and bringing to volume with 50:50 ACN:H₂O.

2.5 PREPARATION OF SPIKE SAMPLES

Spike samples were prepared at F8426 Technical concentrations of 0.125 and 16.0 mg ai/L in both filtered and unfiltered saltwater. The low concentration spike sample (0.125 mg ai/L) for each saltwater was prepared by adding 12.5 µL of the 0.100 mg ai/mL secondary test substance stock solution to a 10.0-mL volumetric flask and bringing to volume with saltwater. The high concentration spike sample (16.0 mg ai/L) for each saltwater was prepared by adding 160 µL of the 1.00 mg ai/mL secondary test substance stock solution to a 10.0-mL volumetric flask and bringing to volume with saltwater.

Each spike level was prepared in triplicate, and a matrix blank was prepared in triplicate from an unfortified aliquot of laboratory saltwater. The high concentration spike samples were diluted with 50:50 ACN:H₂O prior to HPLC analysis to obtain an

instrument response within the calibration range. This preparation method was used with both the filtered and unfiltered saltwater matrix.

2.6 LIQUID CHROMATOGRAPHIC ANALYSIS

The Shimadzu HPLC pump and Shimadzu UV/VIS HPLC detector were set with the following conditions:

Column:	Intersil 5 (C-8) 4.6-mm x 150-mm column
Detector Wavelength:	272 nm
Mobile Phase:	60:40 ACN:H ₂ O, isocratic
Flow Rate:	1.20 mL/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.

2.7 QUANTITATION

The standard response curve (linear regression curve) of F8426 Technical concentration versus peak area (integrator response) was generated from the data obtained during the validation. The equation of the curve (4/23/96) is:

$$\text{mg/L F8426 Technical} = (\text{Peak Area} - 1237.46) / 407391.28,$$

with a correlation coefficient of 1.000. The F8426 Technical concentration found in the samples was calculated using the following equation:

$$\text{mg/L F8426 Technical} = \text{mg/L F8426 Technical from std curve} * \text{dilution factor}$$

2.8 EXAMPLE CALCULATION

Run Date: April 23, 1996

Sample ID: 496469

Response = 48073

Dilution Factor = 1.00

$$\begin{aligned}\text{mg/L F8426 Technical} &= (48073 - 1237.46) / 407391.28 \\ &= 0.115 * 1.00 \\ &= 0.115 \text{ mg ai/L}\end{aligned}$$

2.9 LIMIT OF DETECTION

The limit of detection (LOD) for F8426 Technical was calculated from interpolation of one-half the peak area of the low standard times the dilution factor of the matrix blank. The limit of quantitation (LOQ) was determined from the response of the lowest concentration standard times the dilution factor of the matrix blank.

2.10 ARCHIVES

The final report and all data related to this study will be initially archived at Toxikon Environmental Sciences. Following acceptance of the final report, the raw data and original final report will be sent to FMC Corporation, Princeton, New Jersey, for permanent archiving.